06/02/2022 – uqBio summer school

Scalable, high-speed imaging of molecular biology in action

Douglas Shepherd

Center for Biological Physics
Department of Physics

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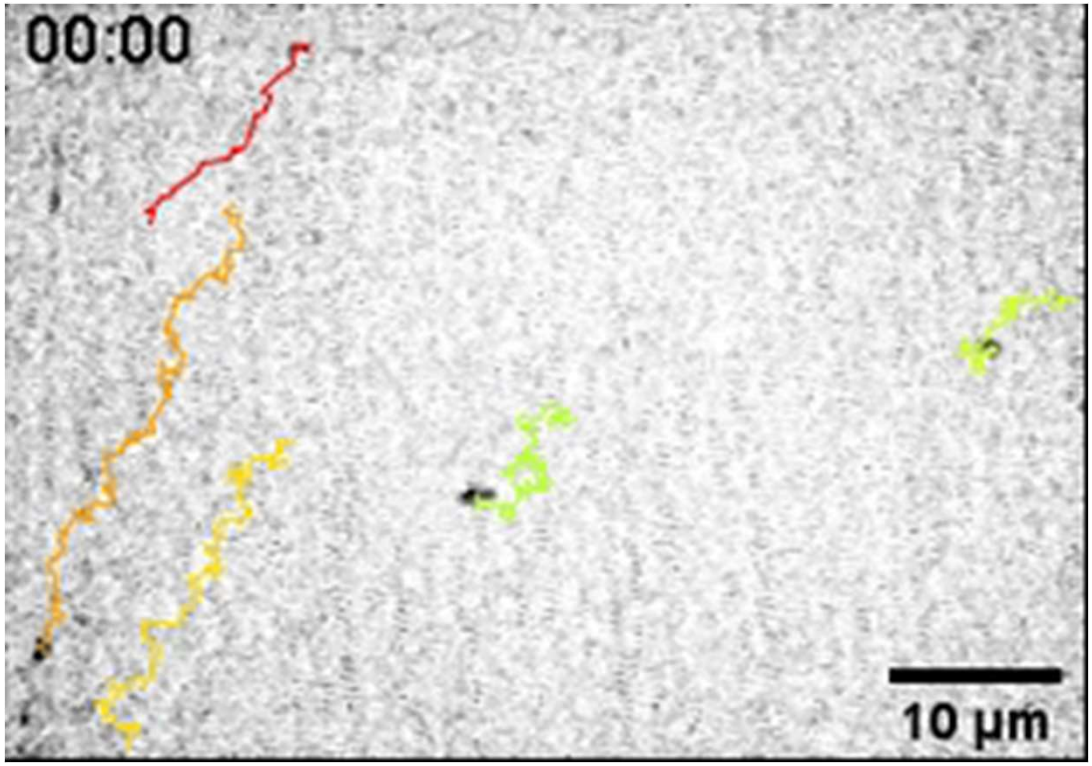


06/02/2022 – uqBio summer school

Scalable, high-speed imaging of molecular biology in action

Franky Djutanta - ASU

Peter Brown - ASU

Rizal Hiriadi - ASU

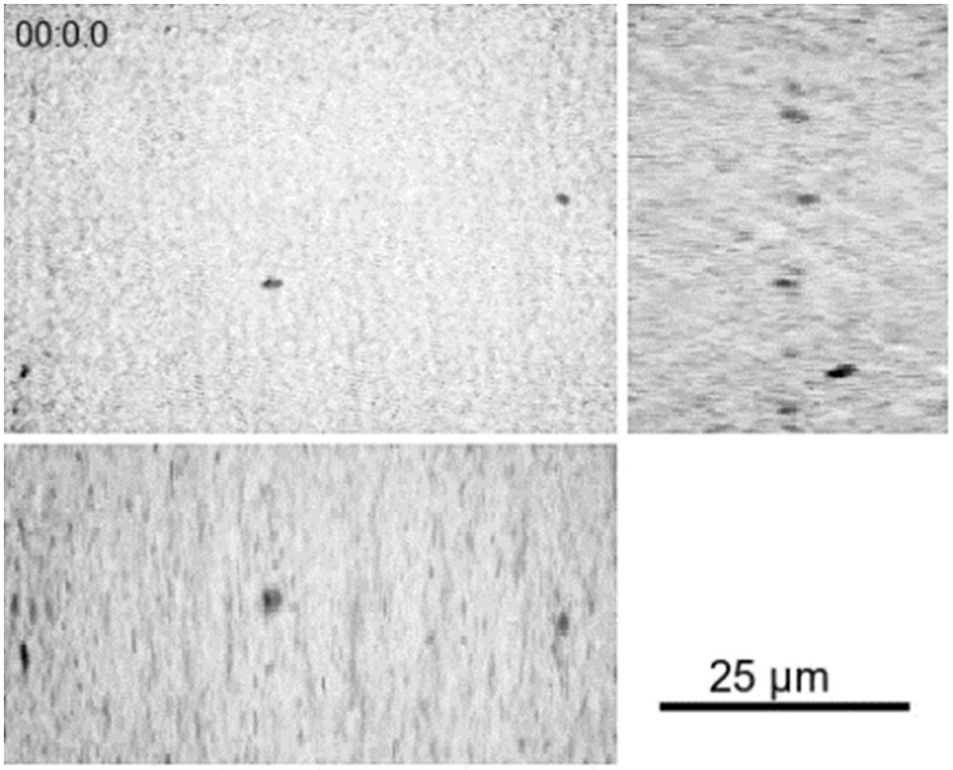
Douglas Shepherd

Center for Biological Physics
Department of Physics

Arizona State University

ASU Center for Biological Physics
Arizona State University

Q|2
Quantitative Imaging and Inference Laboratory



Almost 200 years ago

THE
PHILOSOPHICAL MAGAZINE
AND
ANNALS OF PHILOSOPHY.

[NEW SERIES.]

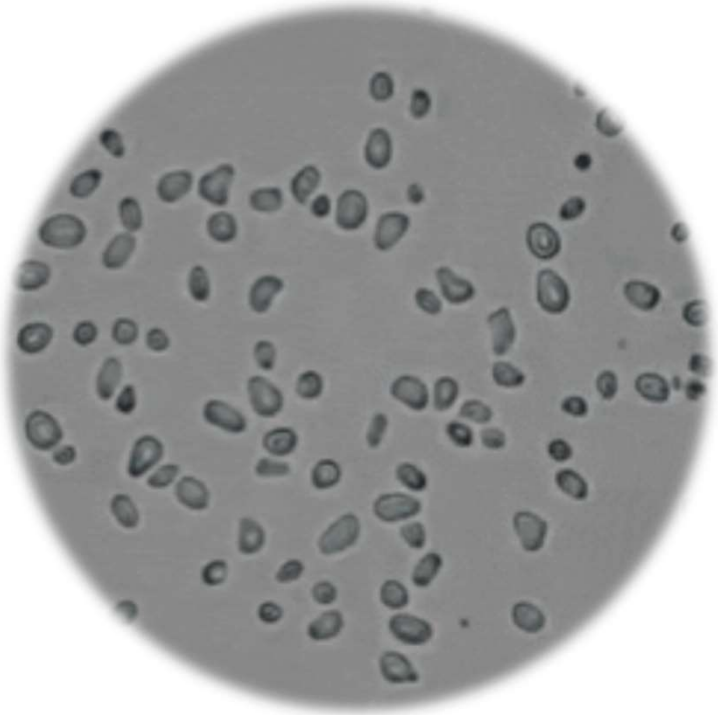
SEPTEMBER 1828.

XXVII. *A brief Account of Microscopical Observations made in the Months of June, July, and August,* 1827, *on the Particles contained in the Pollen of Plants; and on the general Existence of active Molecules in Organic and Inorganic Bodies. By* ROBERT BROWN, *F.R.S., Hon. M.R.S.E. & R.I. Acad., V.P.L.S., Corresponding Member of the Royal Institutes of France and of the Netherlands, &c. &c.*

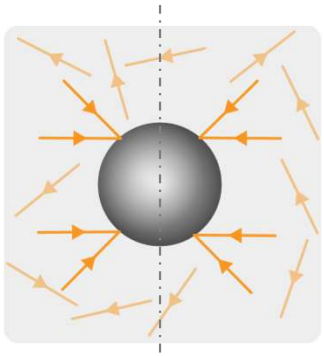
[We have been favoured by the Author with permission to insert the following paper, which has just been printed for private distribution.—ED.]

THE observations, of which it is my object to give a summary in the following pages, have all been made with a simple microscope, and indeed with one and the same lens, the focal length of which is about $\frac{1}{52}$nd of an inch*.

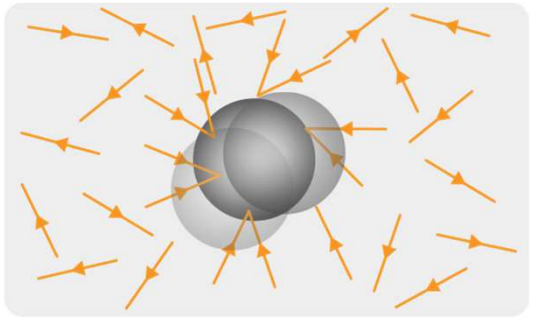
The examination of the unimpregnated vegetable Ovulum, an account of which was published early in 1826†, led me to attend more minutely than I had before done to the structure of the Pollen, and to inquire into its mode of action on the Pistillum in Phænogamous plants.



Brownian motion was first evidence that atoms existed



random motion
caused by molecular collisions



1828 1879 1905 1908


Robert Brown


Charles Darwin


Carl von Nägeli


Albert Einstein

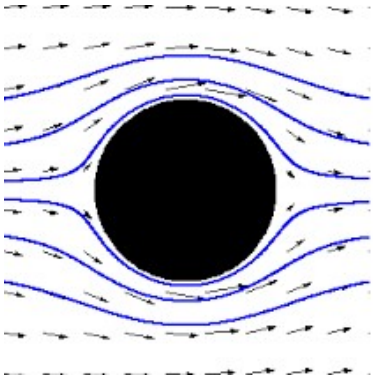

Jean B. Perrin

Hydrodynamic signature written in Brownian motion

Transport phenomena

Brownian motion

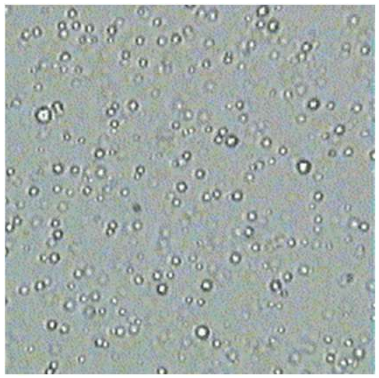
Brownian motion of a sphere (Einstein's PhD)



www.math.armstrong.edu/mmacalc

$$F = A\, v$$

translational drag coefficient



Walker, microscopy-UK

$$A = \frac{k_B T}{D_o}$$

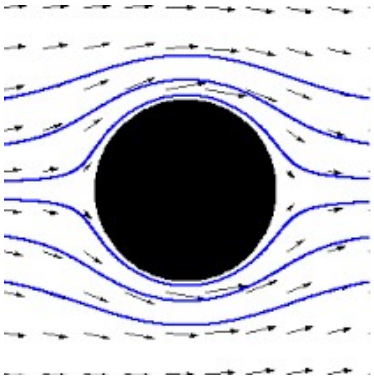
thermal energy

diffusion coefficient

Hydrodynamic signature written in Brownian motion

Brownian motion of a sphere (Einstein's PhD)

Transport phenomena

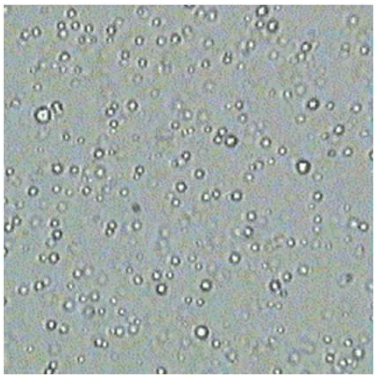


www.math.armstrong.edu/mmacalc

$$F = A\, v$$

translational drag coefficient

Brownian motion



Walker (2015)

$$N_A = D_0(6\pi\eta r)\frac{R}{T}$$

$$A = \frac{k_B T}{D_o}$$

diffusion coefficient

Stokes' theorem

$$D_o = \frac{k_B T}{6\pi\eta r}$$

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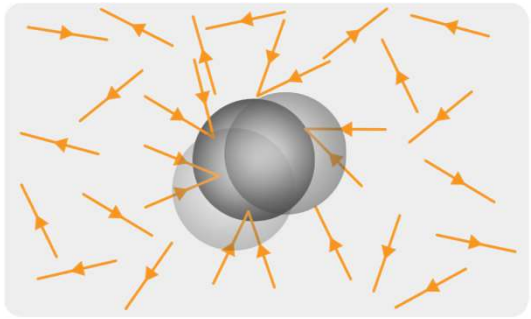
Diffusion coefficients indicate the degree of fluctuation

diffusing spheres along 3D axes

$$\langle |\mathbf{r}(t+\Delta t) - \mathbf{r}(t)|^2 \rangle = 6\, D_o\, \Delta t$$

MSD — diffusion coefficient — interval time

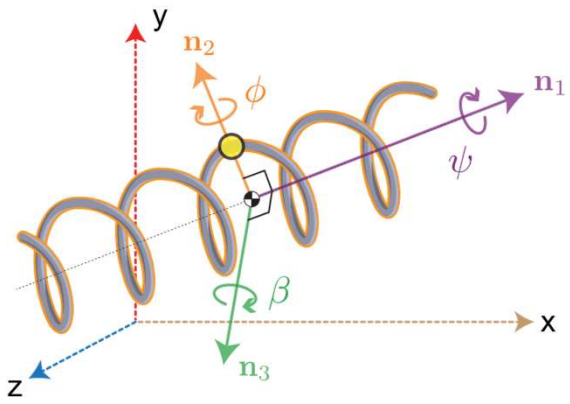
Einstein, 1905 & 1907



arbitrarily-shaped objects along 1D axis

$$\langle |\xi(t+\Delta t) - \xi(t)|^2 \rangle = 2\, D_\xi\, \Delta t$$

MSD / MSAD — diffusion coefficient — interval time

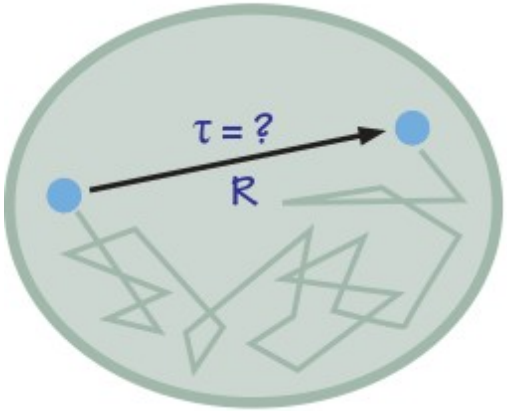


$$\xi = r_{n_1}, r_{n_2}, r_{n_3}$$

$$\xi = \psi, \phi, \beta$$

Given everything we have talked about, how long would it take a particle to randomly explore a cell?

time for protein diffusion across cell



time scale (τ) to traverse distance (R)
given diffusion coefficient (D)

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Mean square displacement (MSD)

ξ_0 ξ_1 ξ_2 ξ_3 ξ_4 ξ_5 ξ_6 ξ_7 ξ_8 ξ_9

$\Delta t = 1$ $\Delta^2_{0,1}$ $\Delta^2_{1,2}$ $\Delta^2_{2,3}$ $\Delta^2_{3,4}$ $\Delta^2_{4,5}$ $\Delta^2_{5,6}$ $\Delta^2_{6,7}$ $\Delta^2_{7,8}$ $\Delta^2_{8,9}$

$\Delta t = 2$ $\Delta^2_{0,2}$ $\Delta^2_{1,3}$ $\Delta^2_{2,4}$ $\Delta^2_{3,5}$ $\Delta^2_{4,6}$ $\Delta^2_{5,7}$ $\Delta^2_{6,8}$ $\Delta^2_{7,9}$

$\Delta^2_{0,3}$ $\Delta^2_{1,4}$ $\Delta^2_{2,5}$ $\Delta^2_{3,6}$ $\Delta^2_{4,7}$ $\Delta^2_{5,8}$ $\Delta^2_{6,9}$

$\Delta^2_{0,4}$ $\Delta^2_{1,5}$ $\Delta^2_{2,6}$ $\Delta^2_{3,7}$ $\Delta^2_{4,8}$ $\Delta^2_{5,9}$

$\Delta^2_{0,5}$ $\Delta^2_{1,6}$ $\Delta^2_{2,7}$ $\Delta^2_{3,8}$ $\Delta^2_{4,9}$

$\Delta^2_{0,6}$ $\Delta^2_{1,7}$ $\Delta^2_{2,8}$ $\Delta^2_{3,9}$

$\Delta^2_{0,7}$ $\Delta^2_{1,8}$ $\Delta^2_{2,9}$

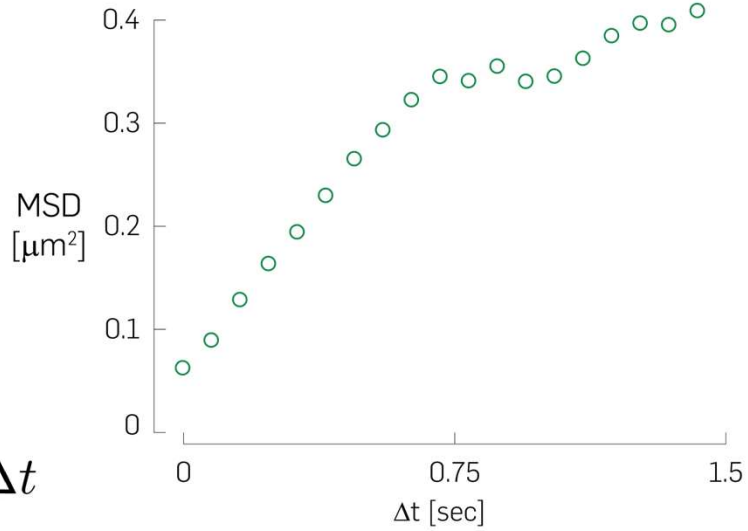
$\Delta^2_{0,8}$ $\Delta^2_{1,9}$

$\Delta t = N - 1$ $\Delta^2_{0,9}$

1. Displacements Δ for each interval time Δt

2. Square each Δ to eliminate sign

3. Take mean for each Δt



MSD $\langle |\xi(t+\Delta t) - \xi(t)|^2 \rangle = 2\ D_\xi\ \Delta t$

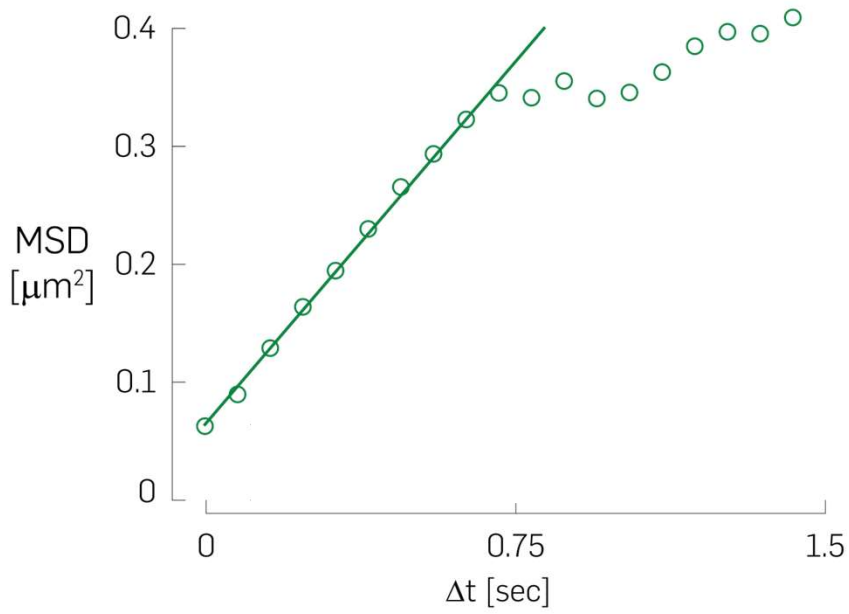
The slope of MSD indicate the diffusion coefficients

$$\langle |\xi(t+\Delta t) - \xi(t)|^2 \rangle = 2\, D_\xi\, \Delta t$$

MSD

diffusion coefficient

interval time



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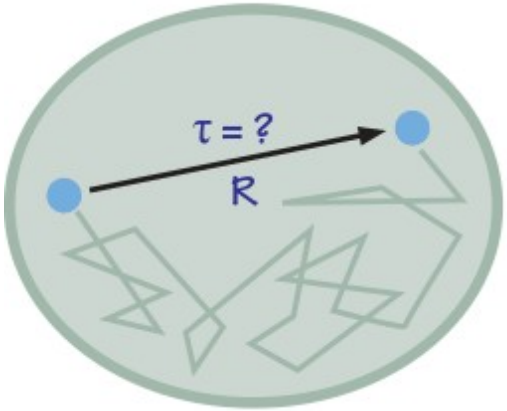


time scale (τ) to traverse distance (R)
given diffusion coefficient (D)

$\tau = R^2/6D$

protein in cytoplasm $D \approx 10 \frac{\mu m^2}{s}$

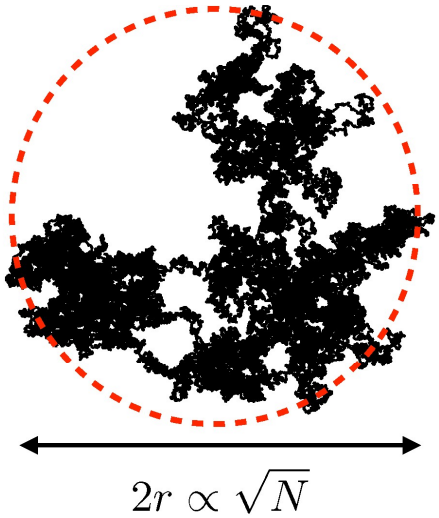
E. coli, $R \approx 1\ \mu m \Longrightarrow \tau \approx 10\ ms$

HeLa cell, $R \approx 20\ \mu m \Longrightarrow \tau \approx 10\ s$

neuronal cell axon, $R \approx 1\ cm \Longrightarrow \tau \approx 10^6\ s \approx 20\ days!$

Physical Biology of the Cell, 2nd edition

Number of distinct sites visited by unbiased random walks



Total number of sites inside explored region after *N* steps

1D $N_{\mathrm{tot}} \propto \sqrt{N}$

2D $N_{\mathrm{tot}} \propto N$

3D $N_{\mathrm{tot}} \propto N\sqrt{N}$

In 1D and 2D every site gets visited after a long time

In 3D some sites are never visited even after a very long time!

Shizuo Kakutani: "A drunk man will find his way home, but a drunk bird may get lost forever."

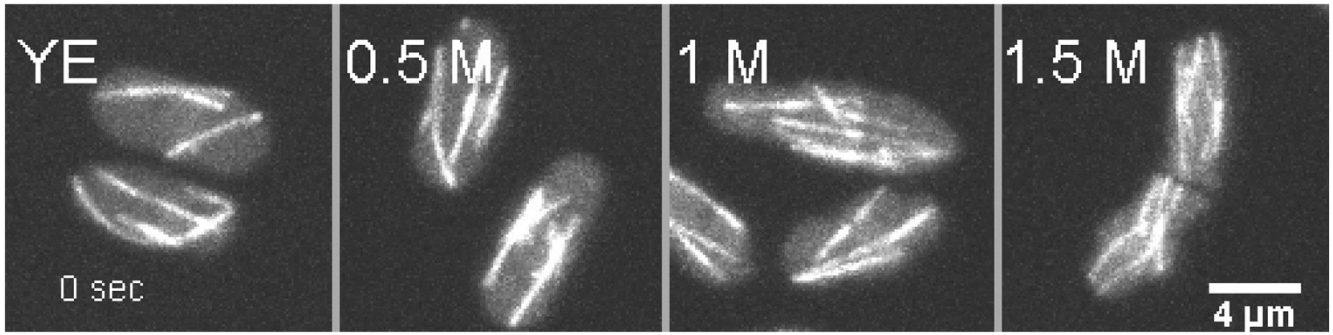
Number of distinct visited sites after *N* steps

1D $N_{\mathrm{vis}} \approx \sqrt{8N/\pi}$

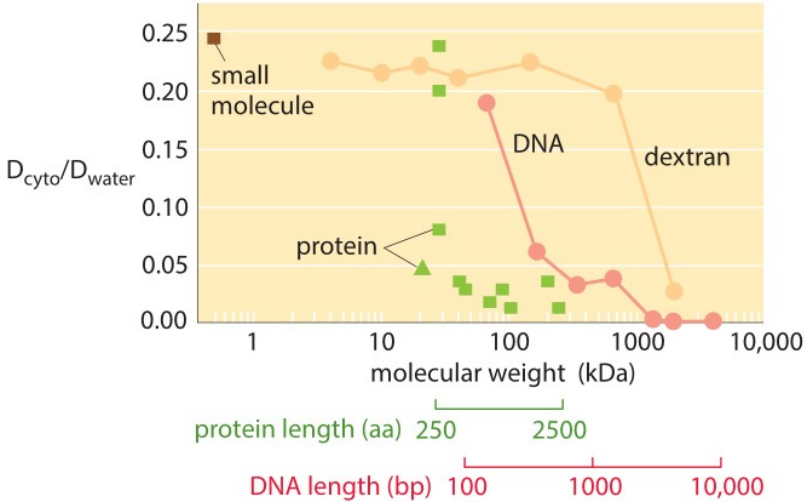
2D $N_{\mathrm{vis}} \approx \pi N/\ln(8N)$

3D $N_{\mathrm{vis}} \approx 0.66N$

What about in a crowded environment?

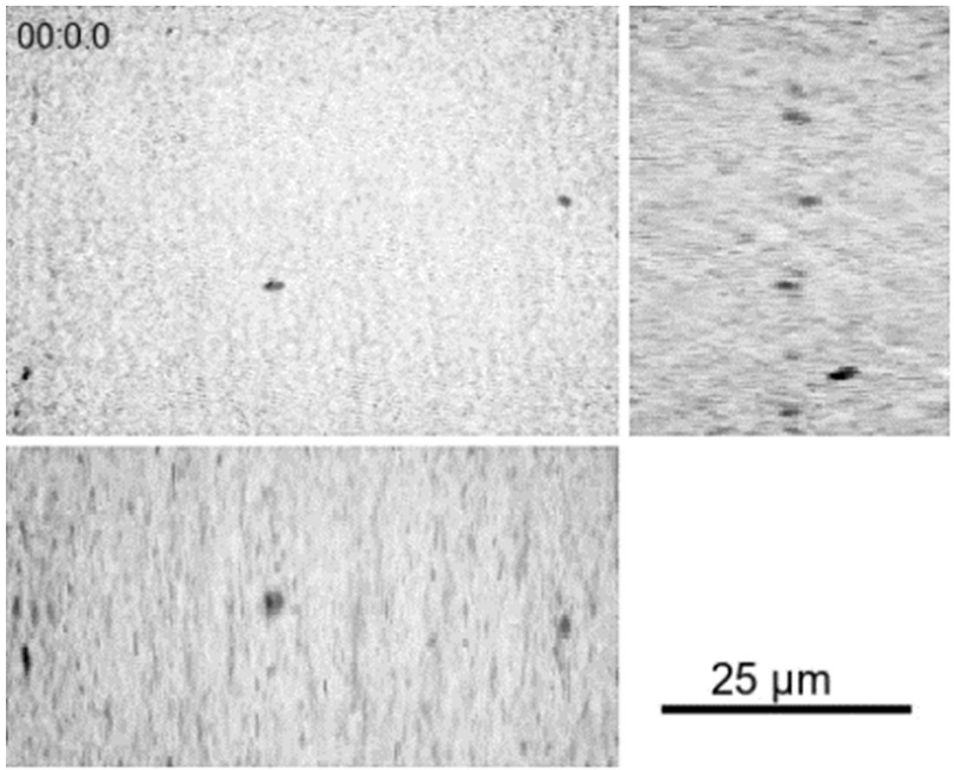


Physical Biology of the Cell, 2nd edition



Physical Biology of the Cell, 2nd edition

Single particle tracking requires a time series of images



Single particle tracking of fluid flows

Vol. 37, No. 1 Transactions, American Geophysical Union February 1956

The Rate of Dissipation of Energy and the Energy Spectrum in a Low-Speed Turbulent Jet

WAN-CHENG CHIU AND LOUIS N. RIB

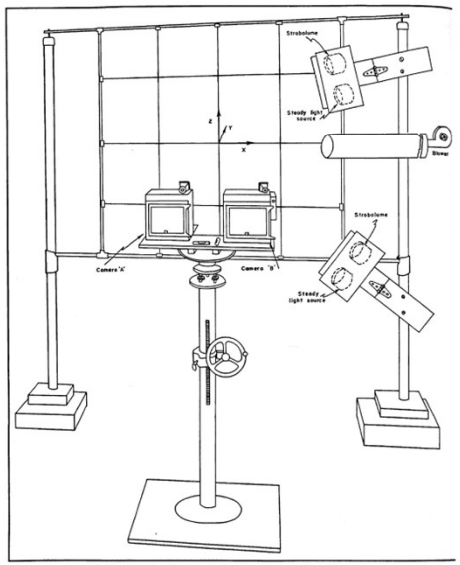


FIG. 1 – Schematic diagram of apparatus, field of observation, and grid

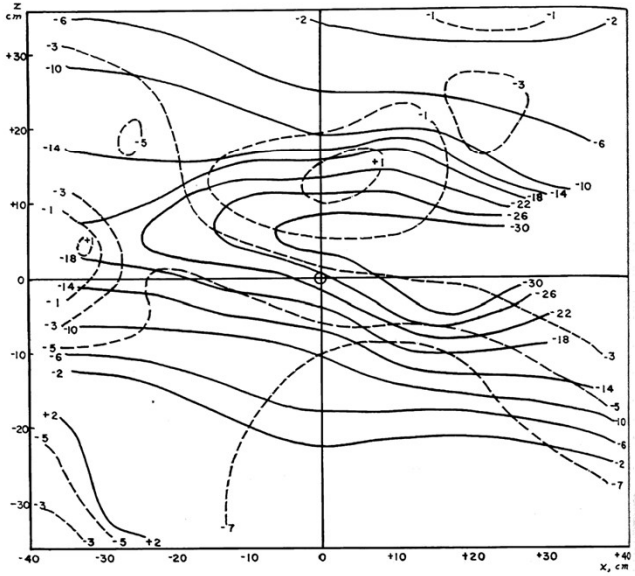
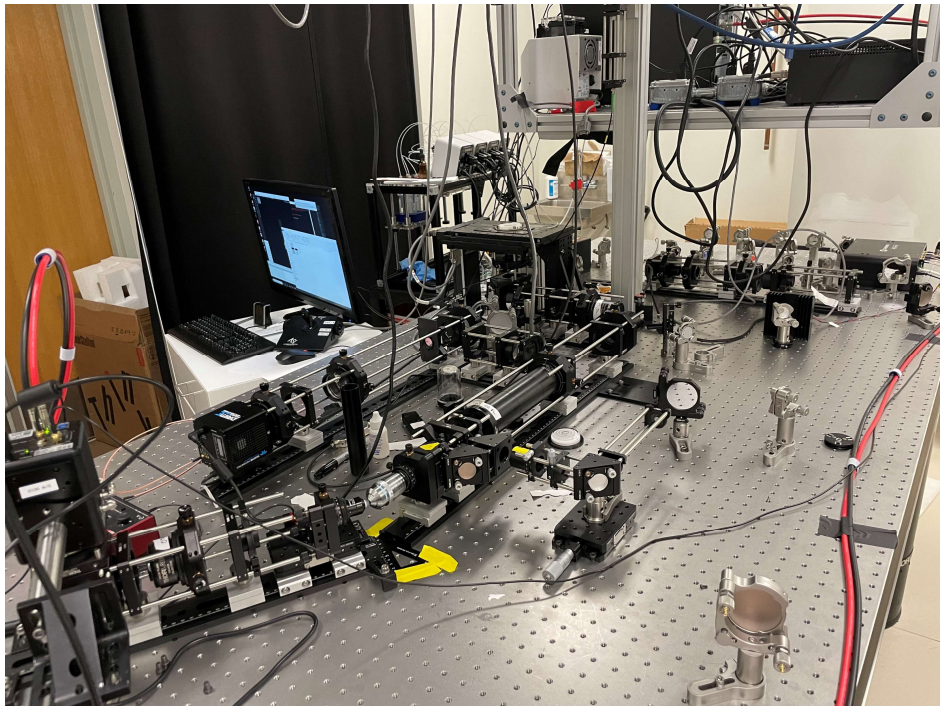


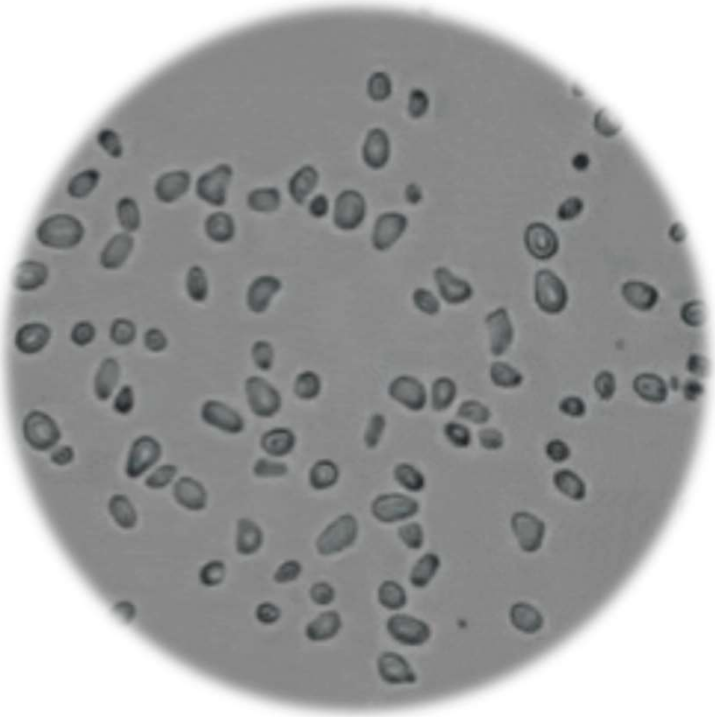
FIG. 2 – The $x - z$ plane at $y = 18$ cm showing the analyzed field of mean motion; the solid lines are the $\bar{u}$ component and the dashed lines are the $\bar{v}$ component, cm/sec

Microscopes have evolved, all based on the same underlying physics



10.1119/1.10903
10.7554/eLife.57681
BIONICS and qi2lab - unpublished

Contrast mechanism is just as critical



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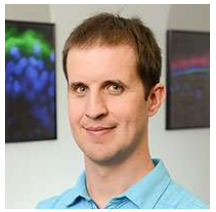
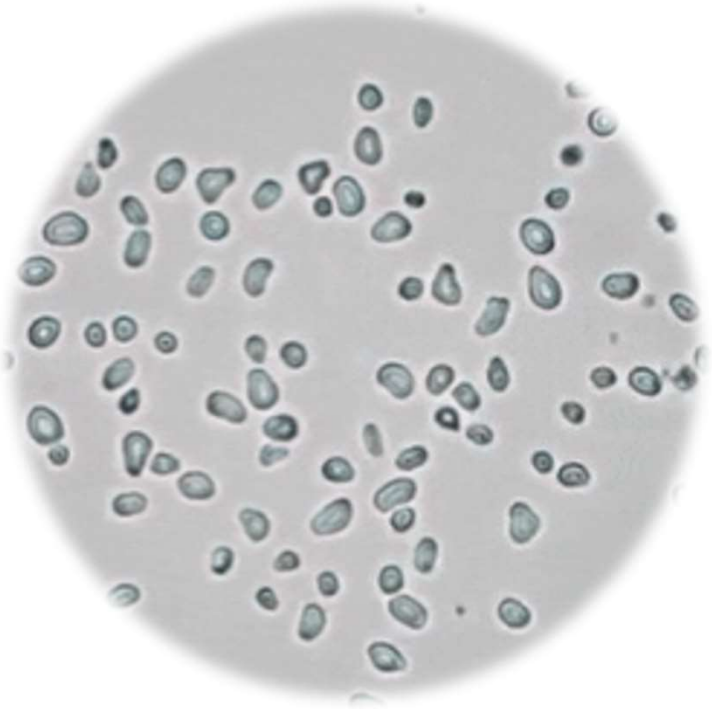
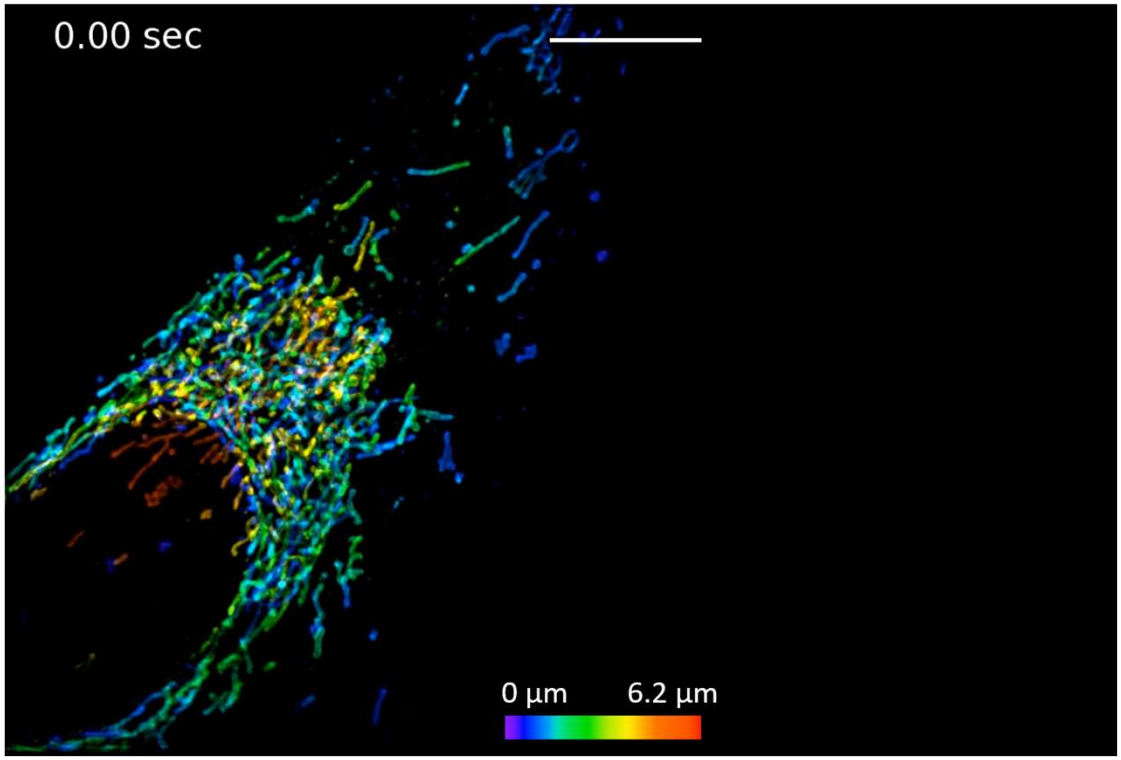


Reto Fiolka
UTSW





10.7554/eLife.57681
10.1101/2022.05.19.492671

Center for Biological Physics
Arizona State University

Advancements in molecular labeling and optical microscopy enable quantitative tracking

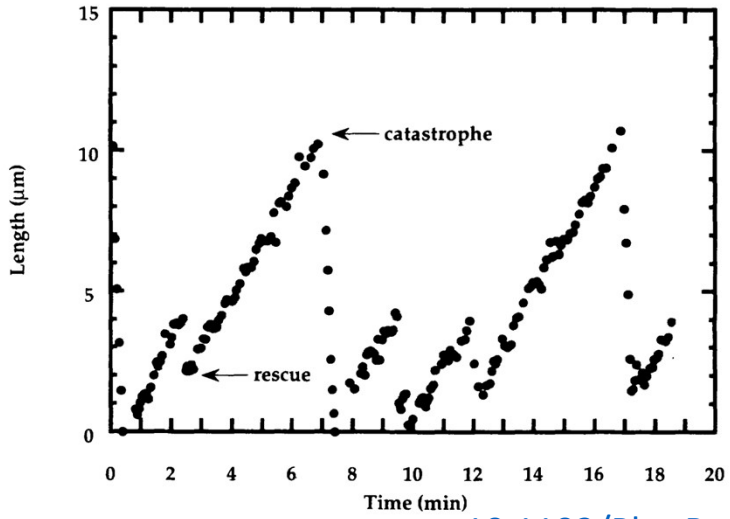
Dynamic instability of microtubule growth

Tim Mitchison & Marc Kirschner

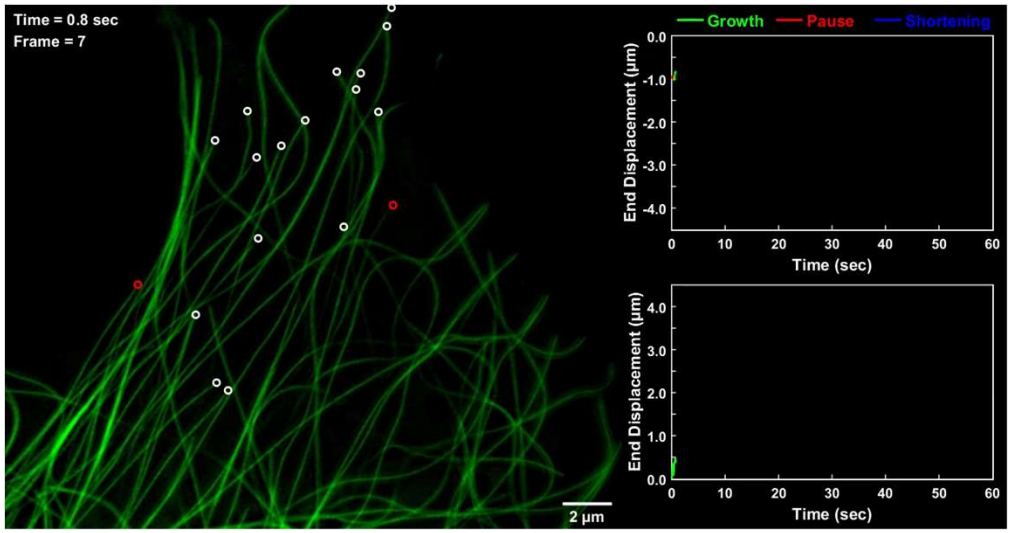
Department of Biochemistry and Biophysics, University of California at San Francisco, San Francisco, California 94143, USA

We report here that microtubules in vitro *coexist in growing and shrinking populations which interconvert rather infrequently. This dynamic instability is a general property of microtubules and may be fundamental in explaining cellular microtubule organization.*

10.1038/312237a0



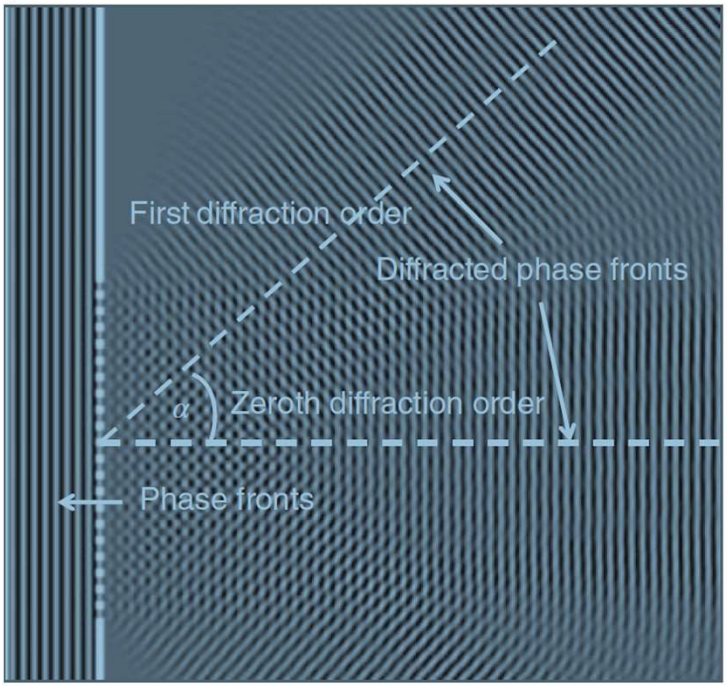
10.1103/PhysRevE.50.1579

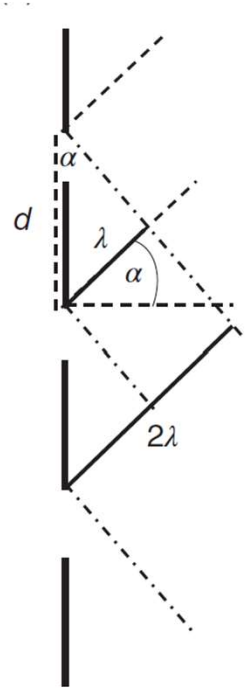


10.1016/j.cell.2018.09.057

Wave nature of light

Interference





10.1002/9783527687732

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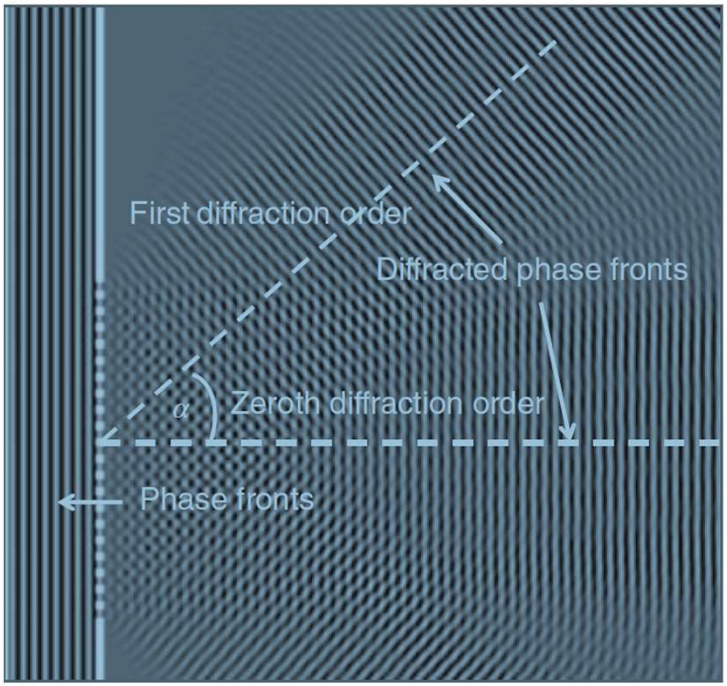
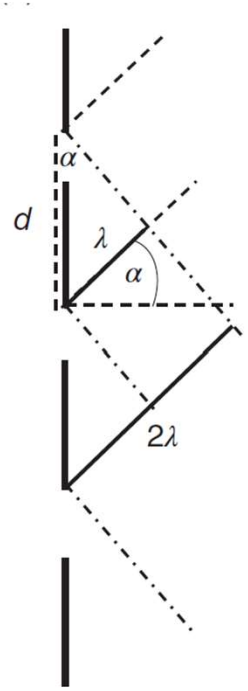
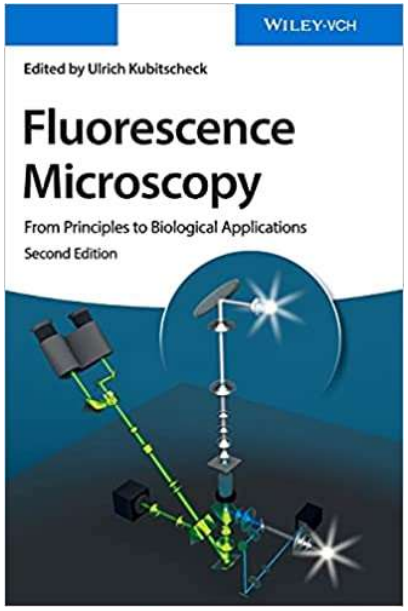
Wave nature of light

Interference







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Wave nature of light

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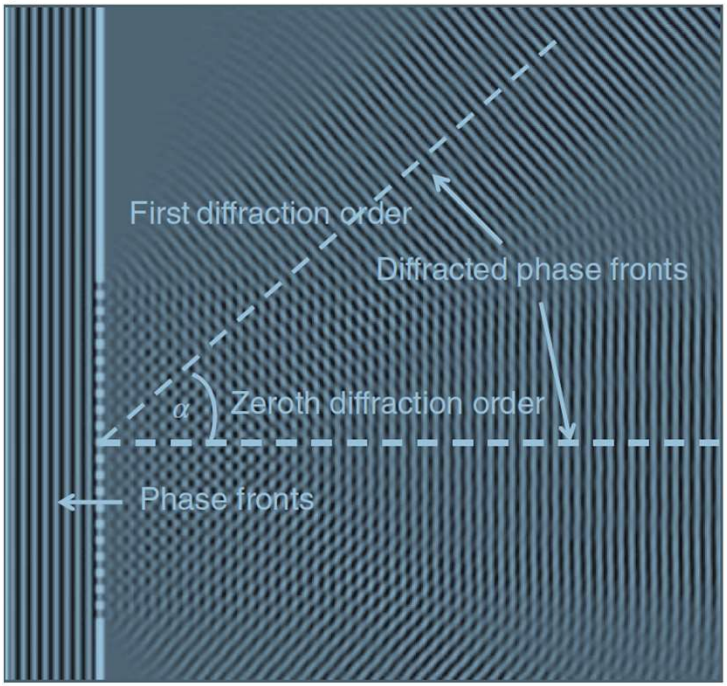
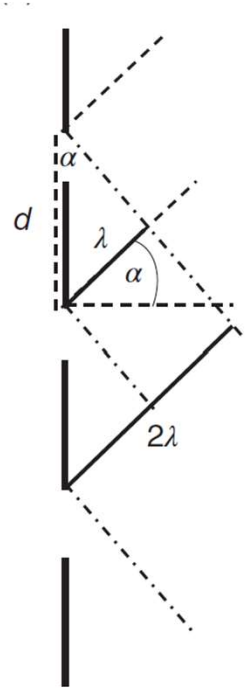
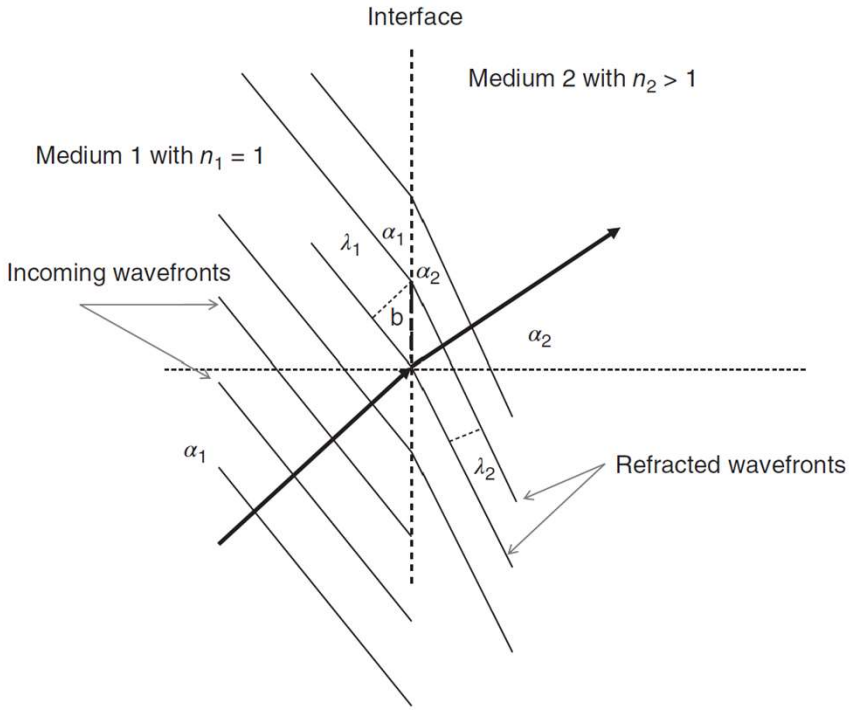




Refraction and refractive index



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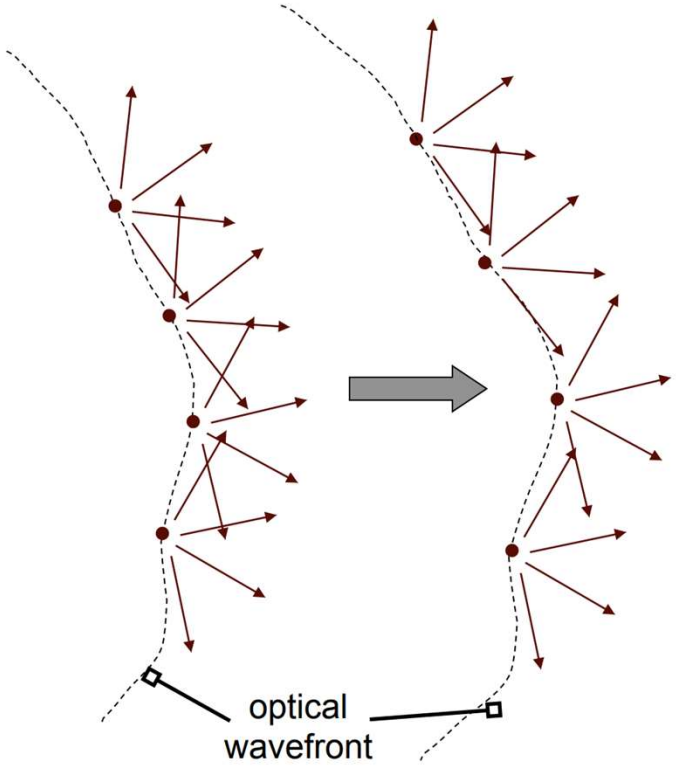
Propagation of waves through optical system



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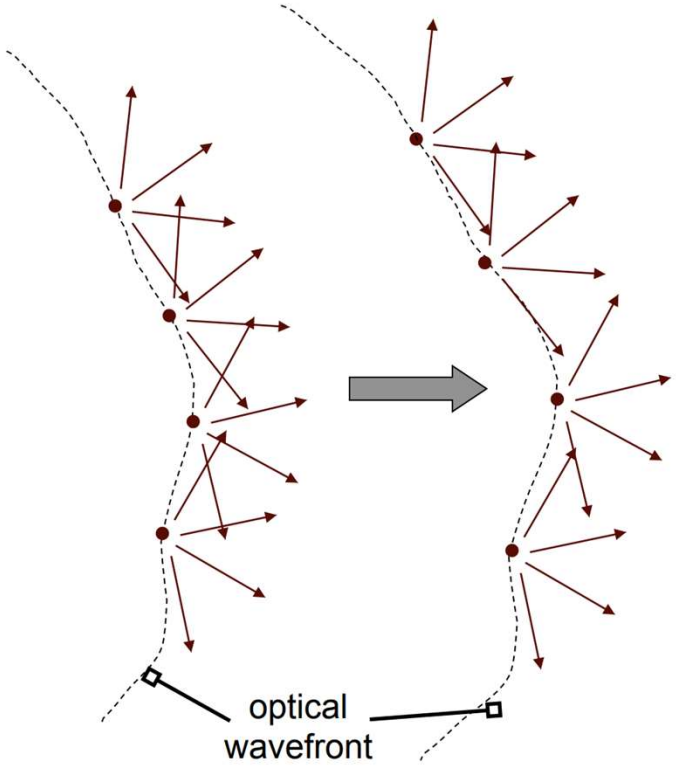

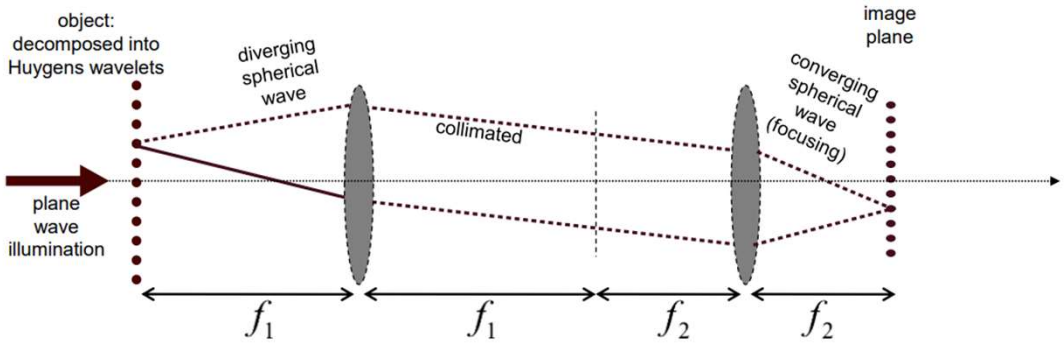

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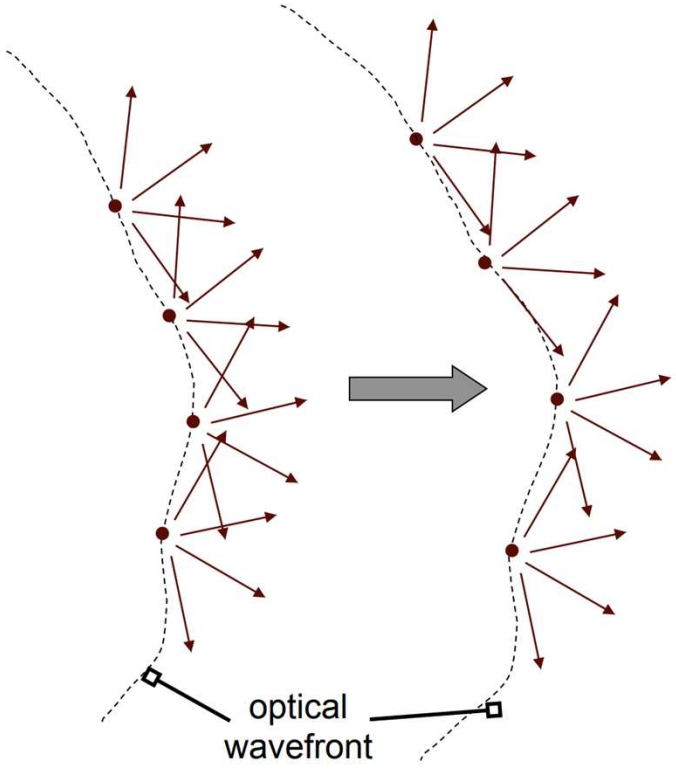
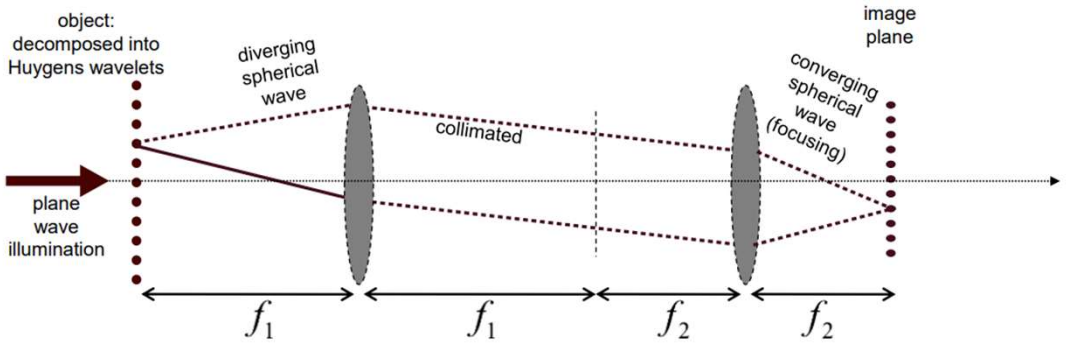
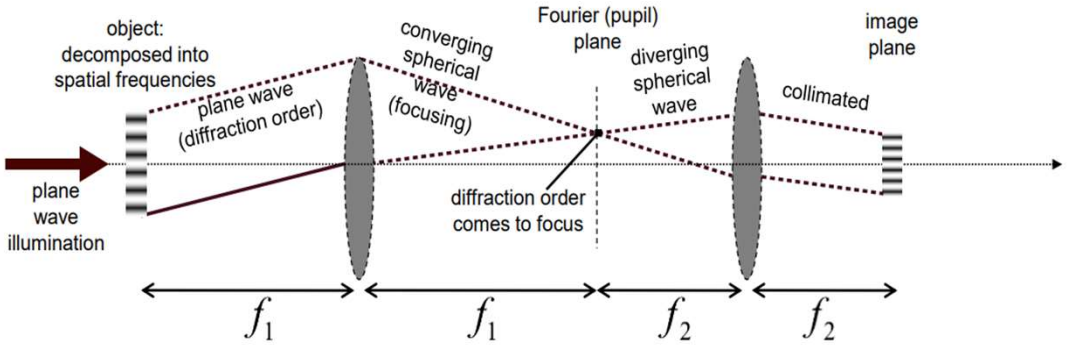
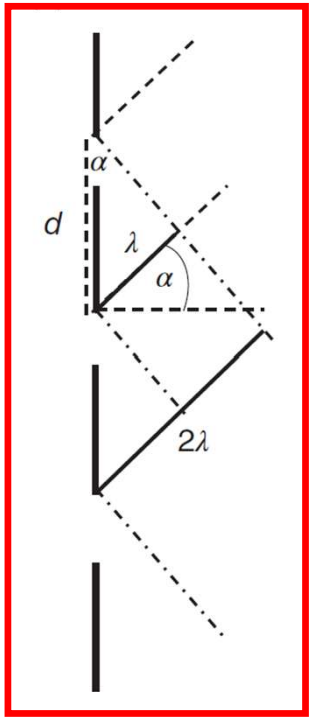






10.1002/9783527687732

Optical microscope as a measurement tool



Bragg condition

$$d\sin(\alpha_n) = n\lambda$$

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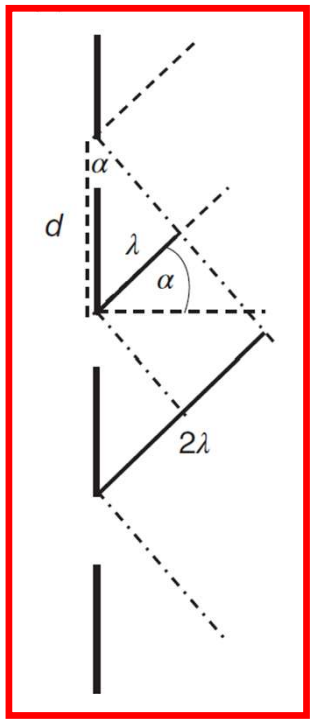
Optical microscope as a measurement tool



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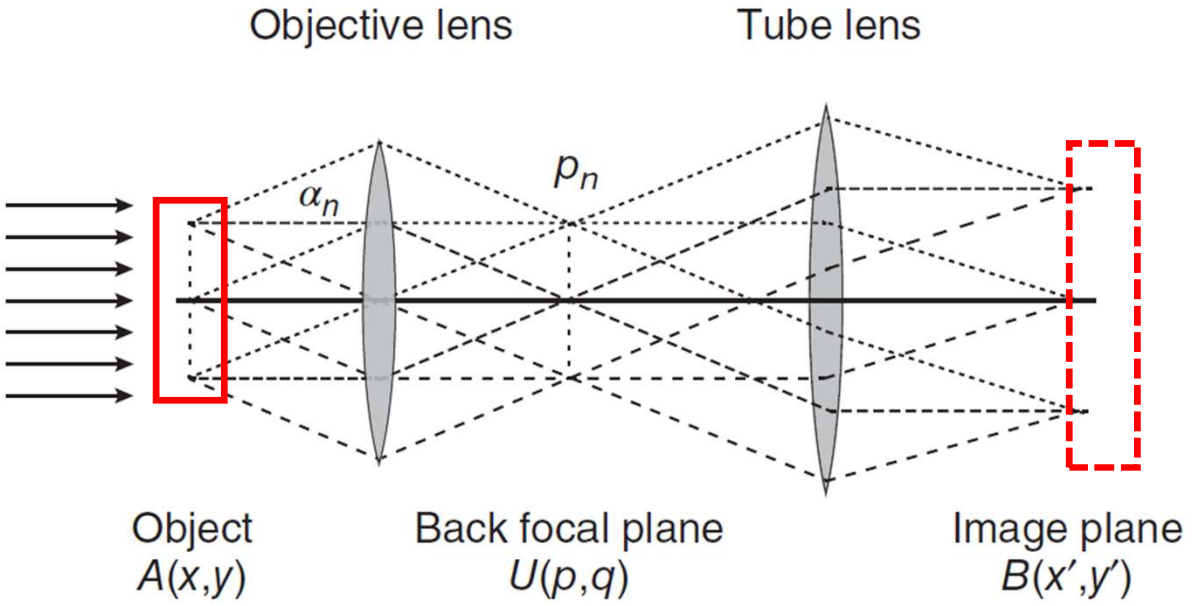

von Bieren condition

$$\sin(\alpha_n) = \frac{p_n}{f}$$

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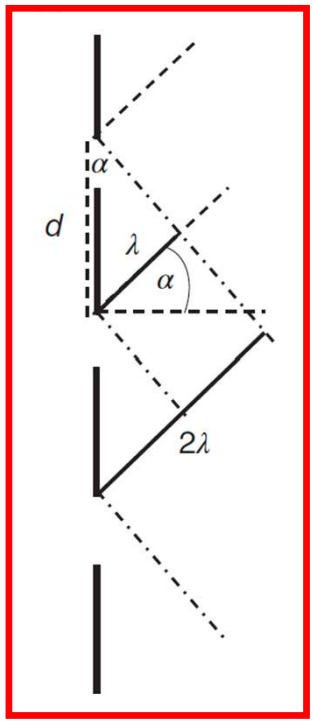
Optical microscope as a measurement tool



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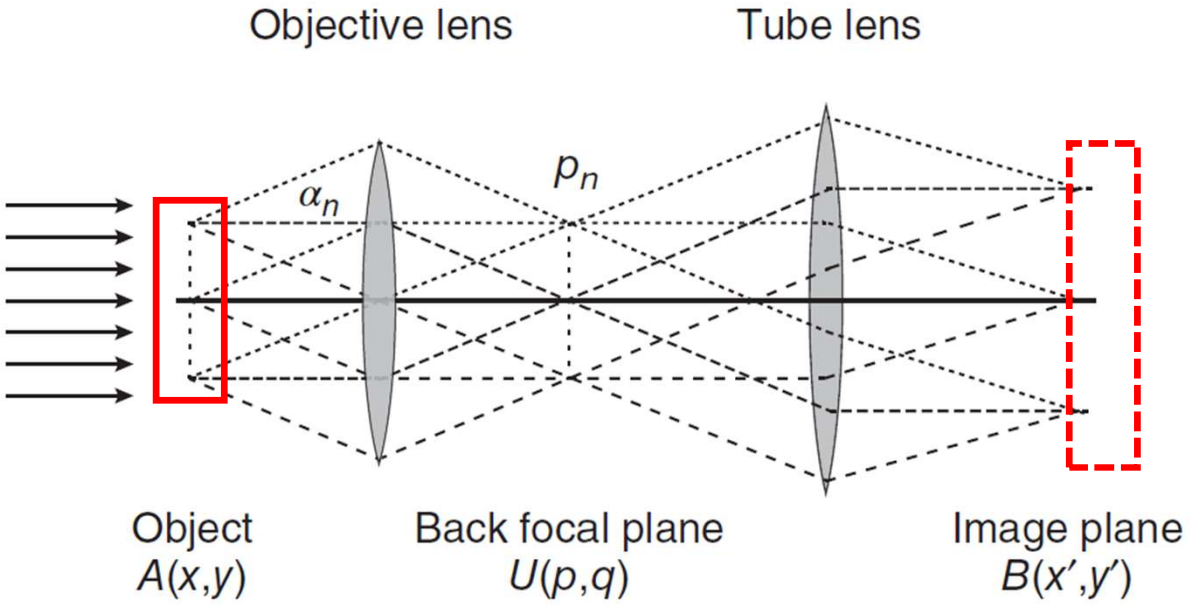

von Bieren condition

$$\sin(\alpha_n) = \frac{p_n}{f}$$

Measured grating spacing

$$d = \frac{n\lambda f}{p_n}$$

Resolution limit

$$d_{min} > \frac{\lambda_0}{n\sin(\alpha_{max})}$$

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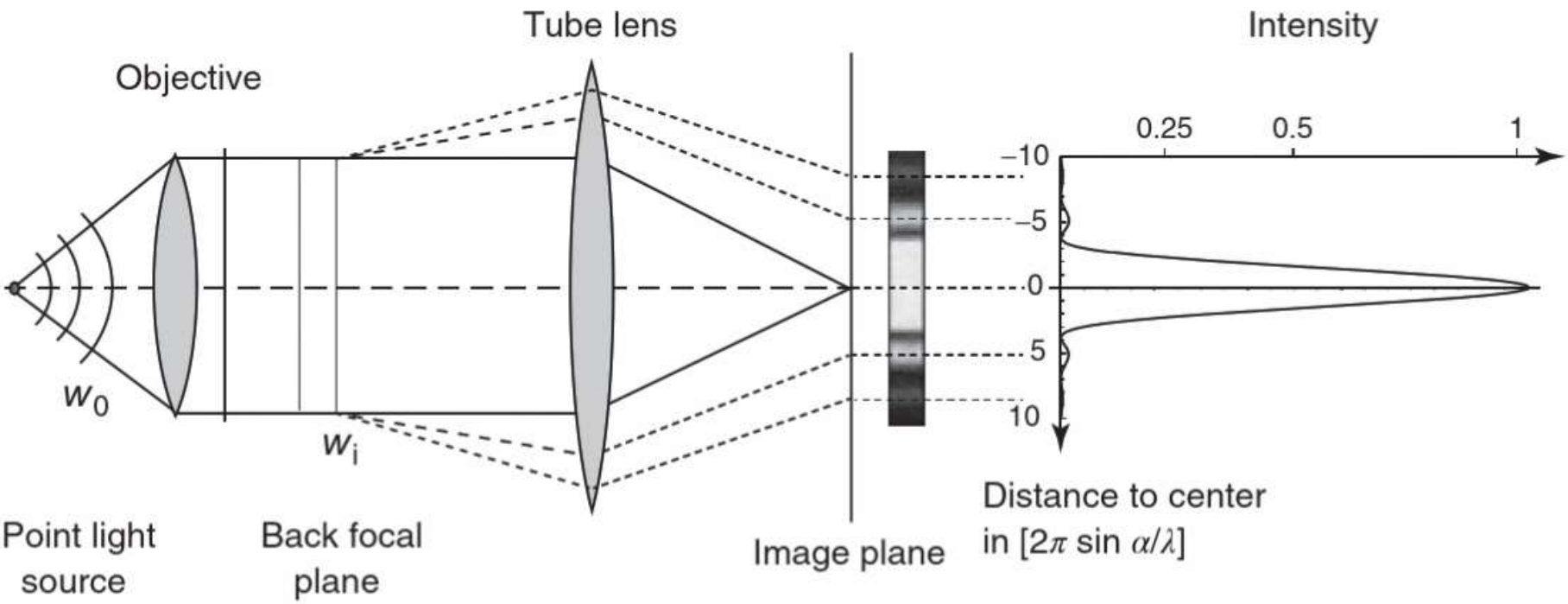
Optical microscopes are band limited



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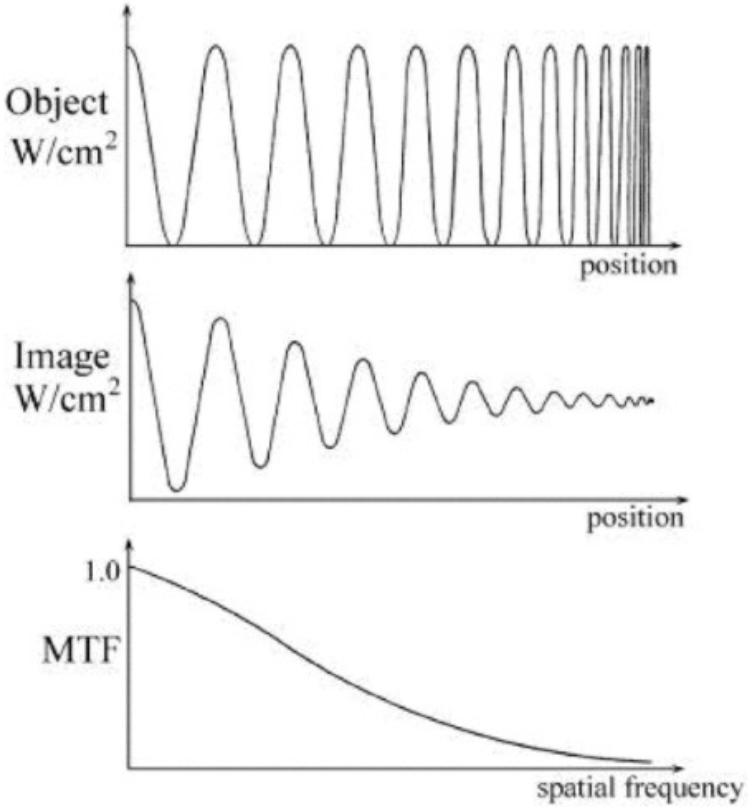
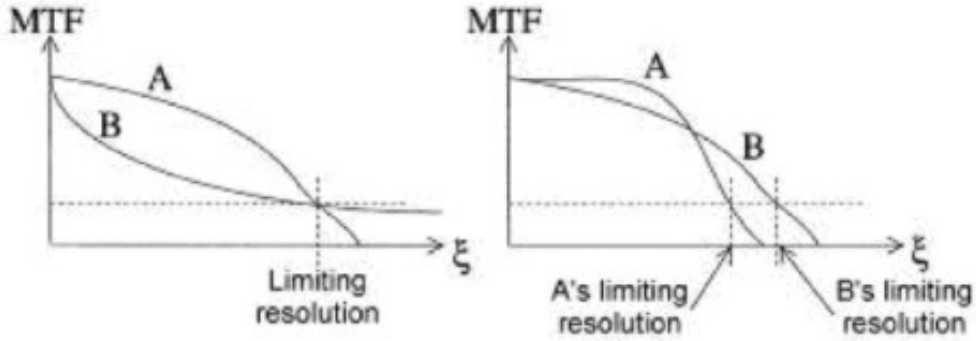
The *transfer function* offers a holistic quantification of optical microscope performance





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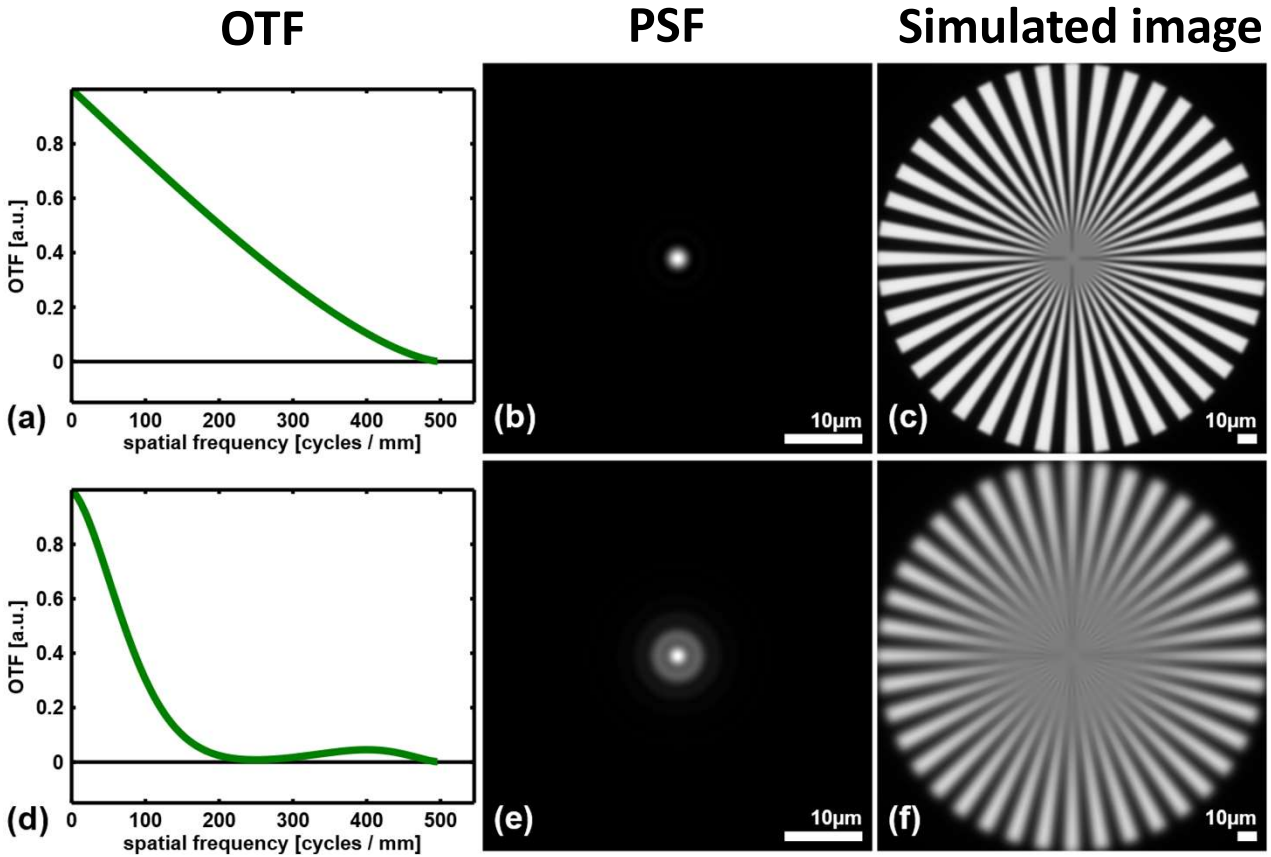
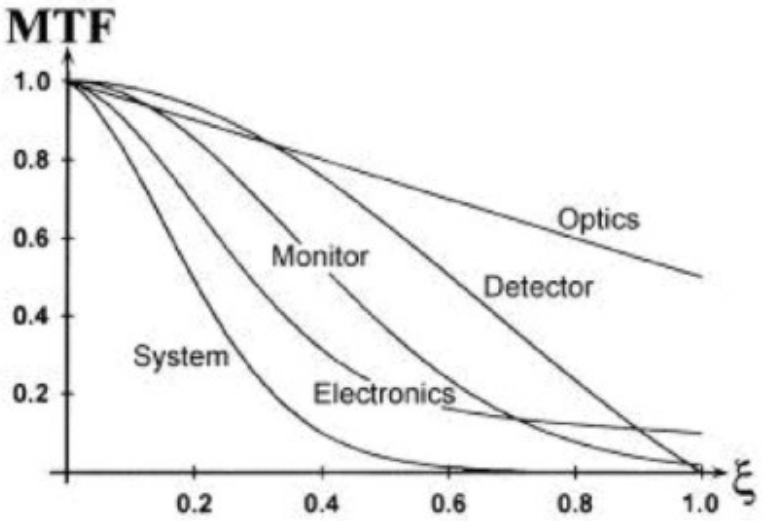
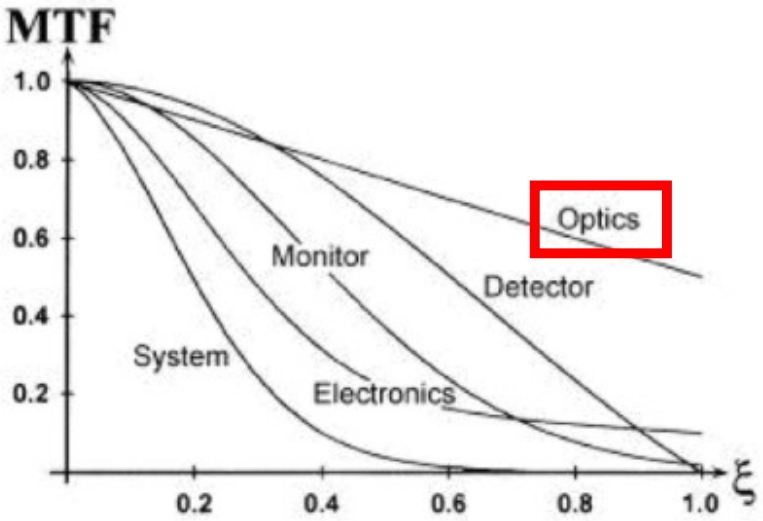


Overall transfer function depends on all components

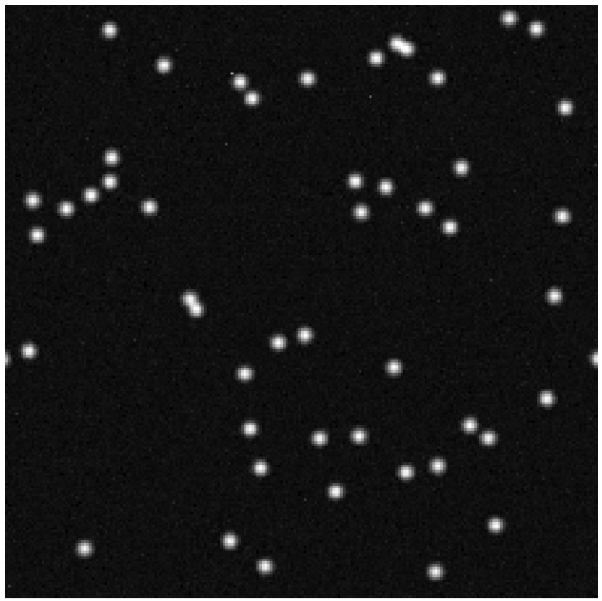


10.1002/9783527687732
Hamamatsu camera simulator

Overall transfer function depends on all components



No noise



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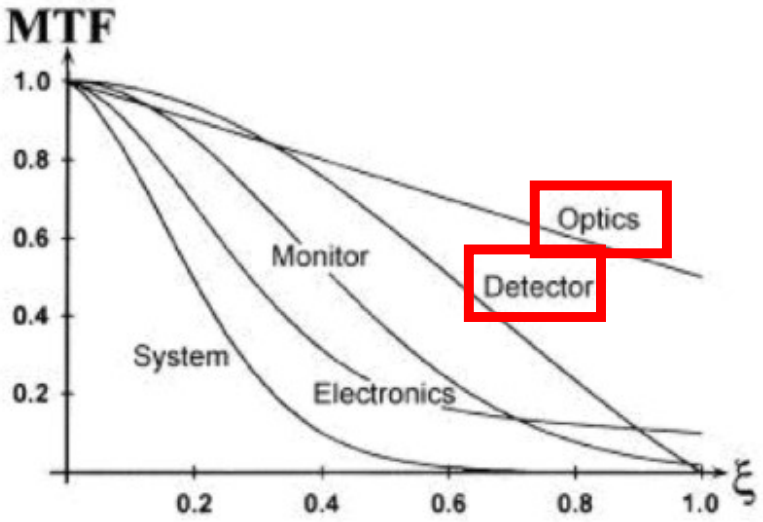
Overall transfer function depends on all components



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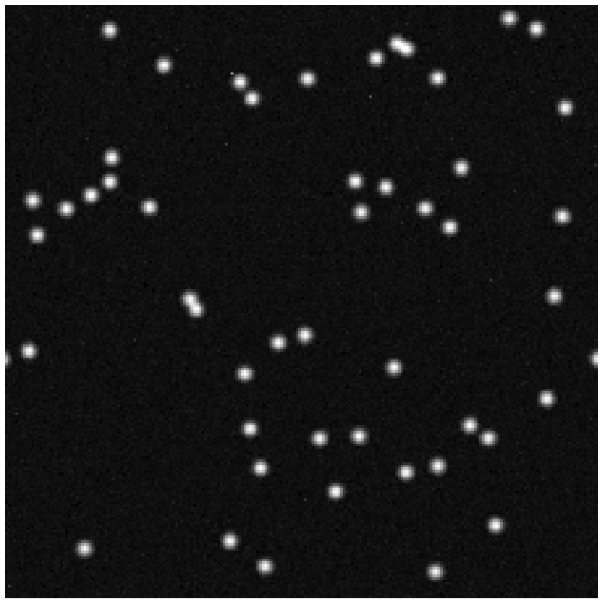
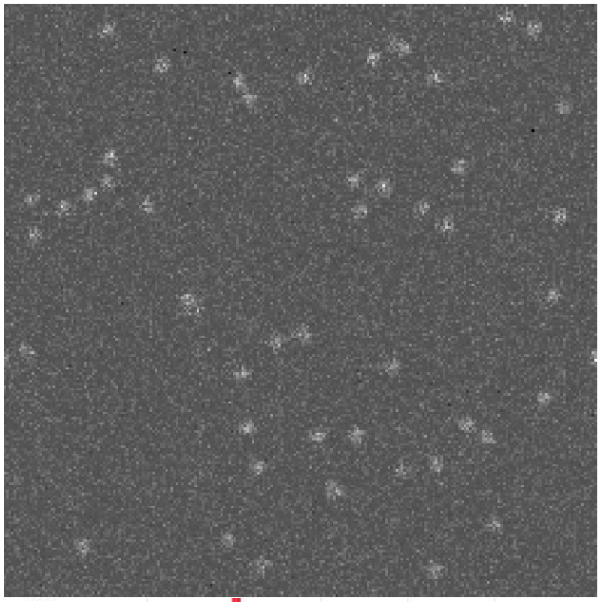
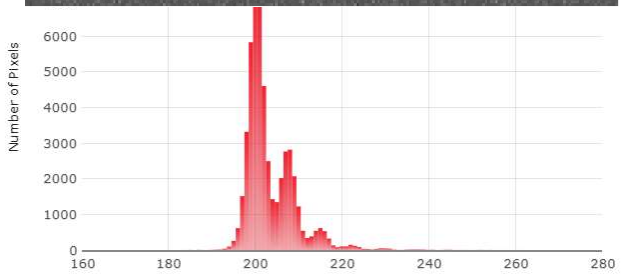


Poisson photon noise
+
<0.5 e- read-noise





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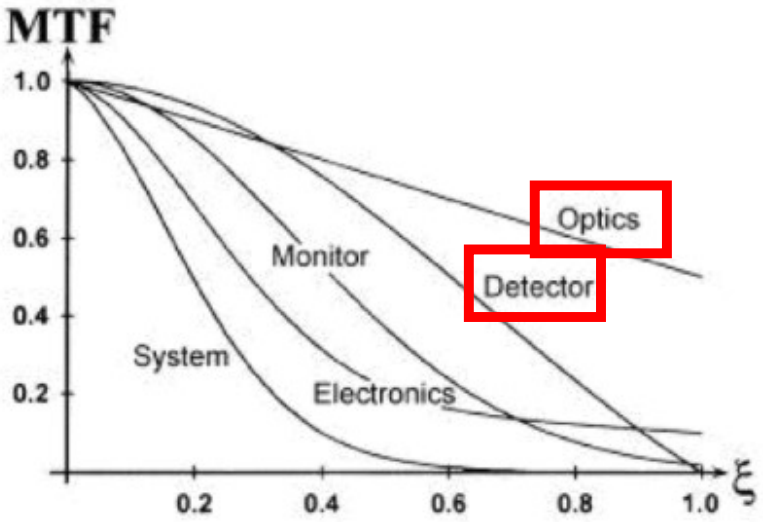
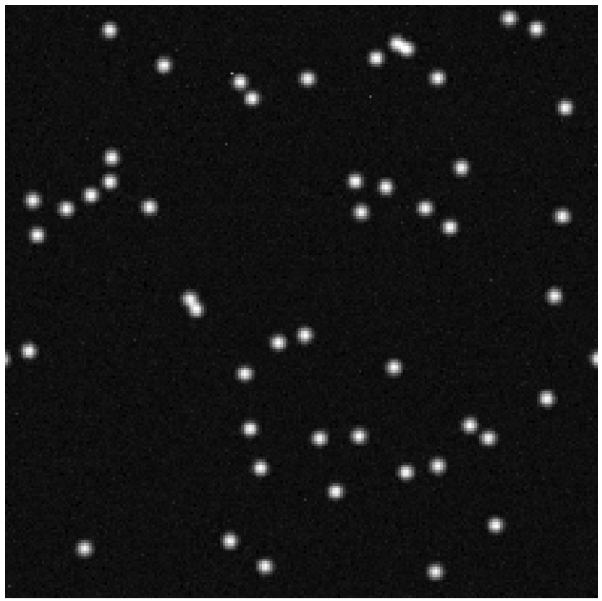


No noise



Poisson photon noise
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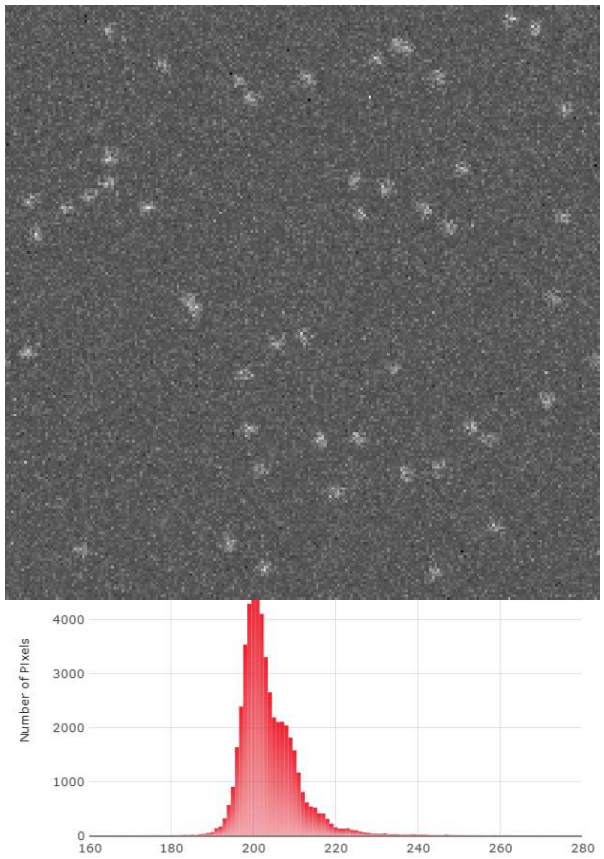
>1 e- read-noise



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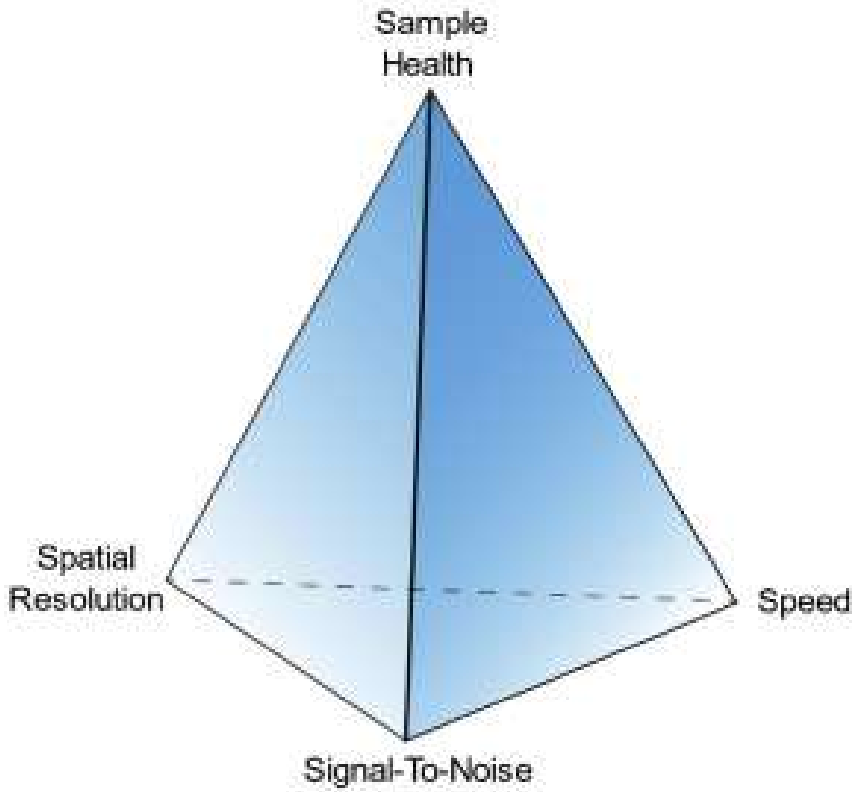
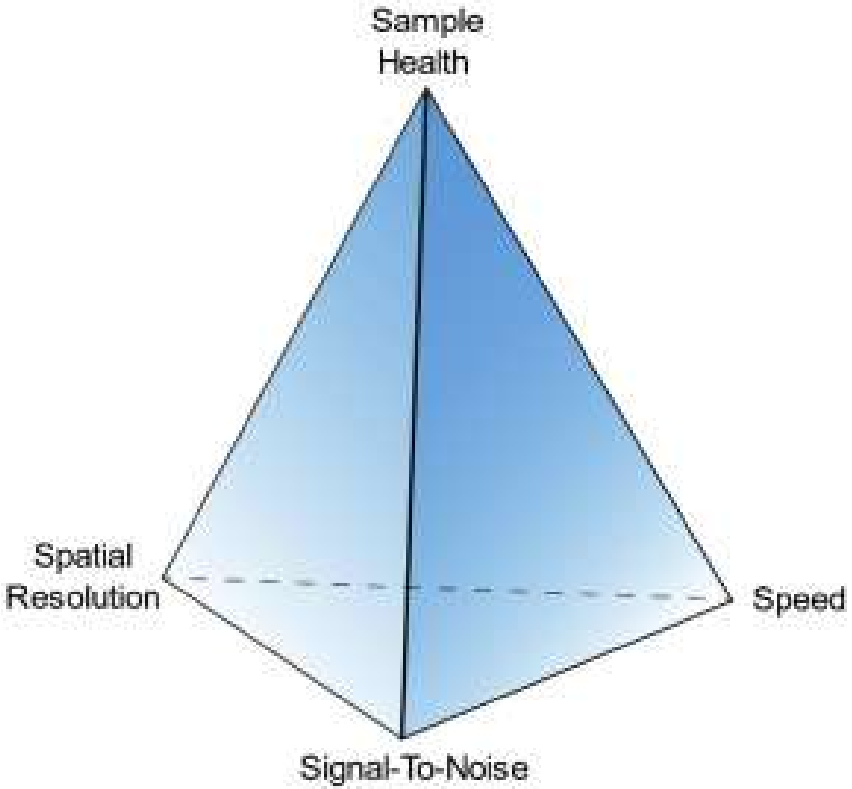
Hamamatsu camera simulator

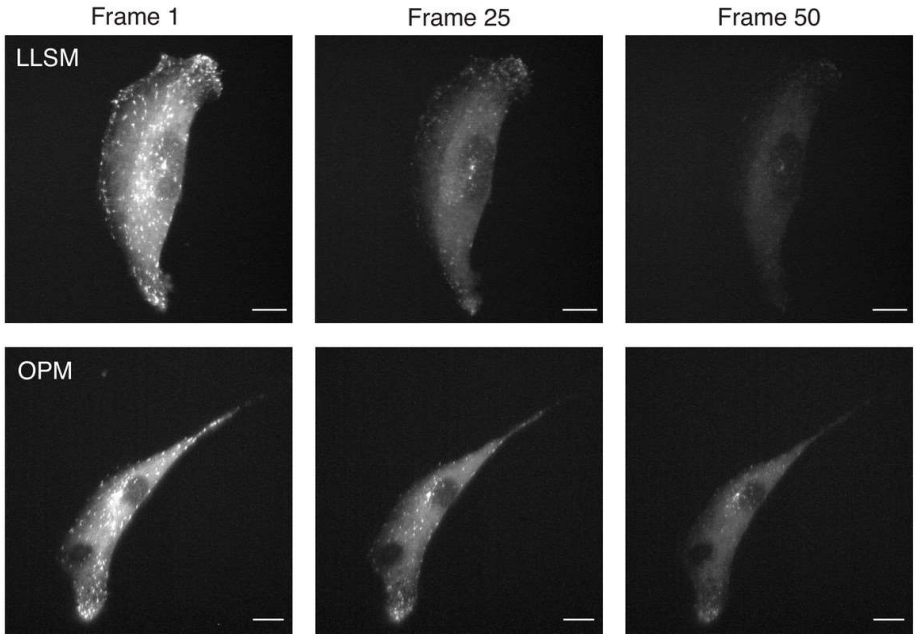
Fluorescence microscopy has a fundamental tradeoff between contrast, resolution, and speed



10.1016/j.ceb.2020.04.008

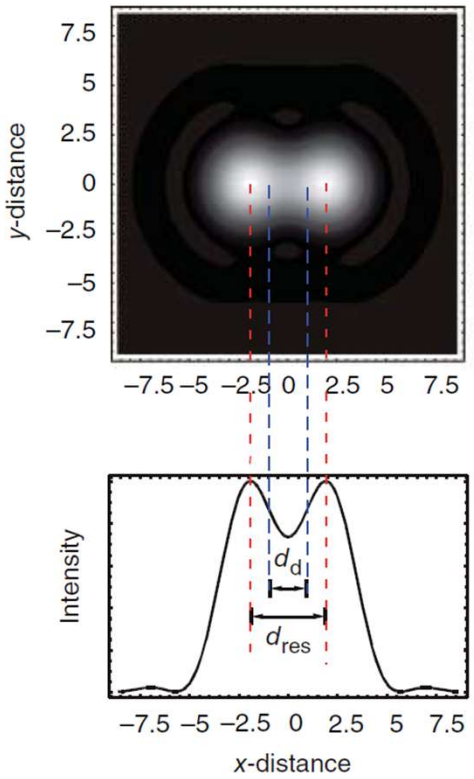
Fluorescence has a limited photon budget

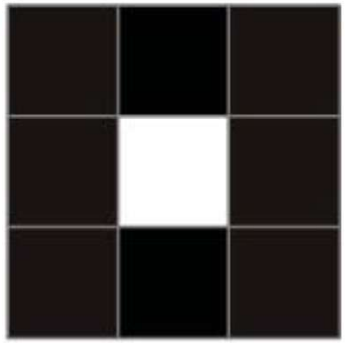




10.7554/eLife.57681
10.1016/j.ceb.2020.04.008

Digital sampling can further limit information





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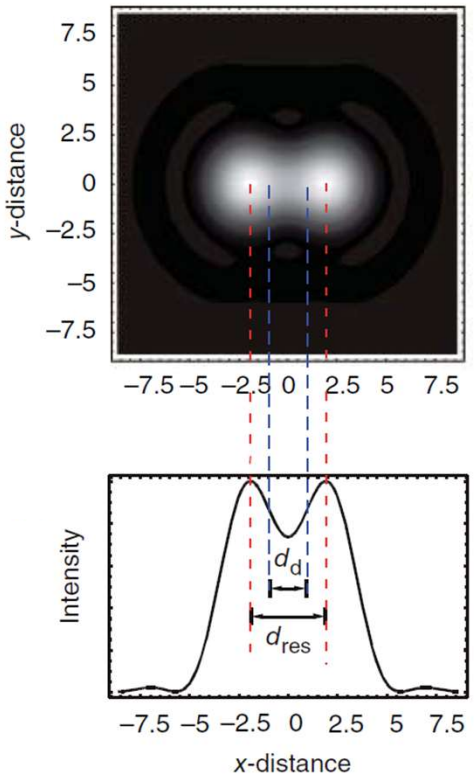

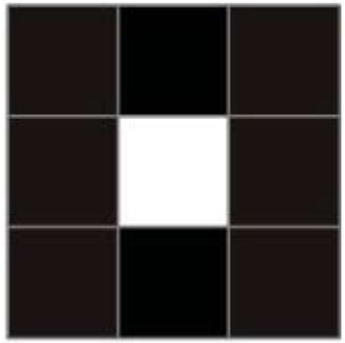

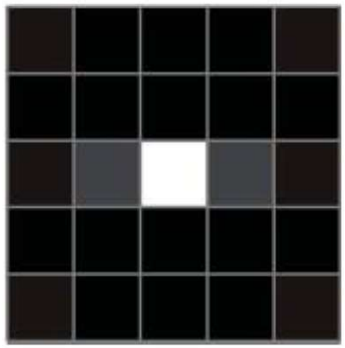

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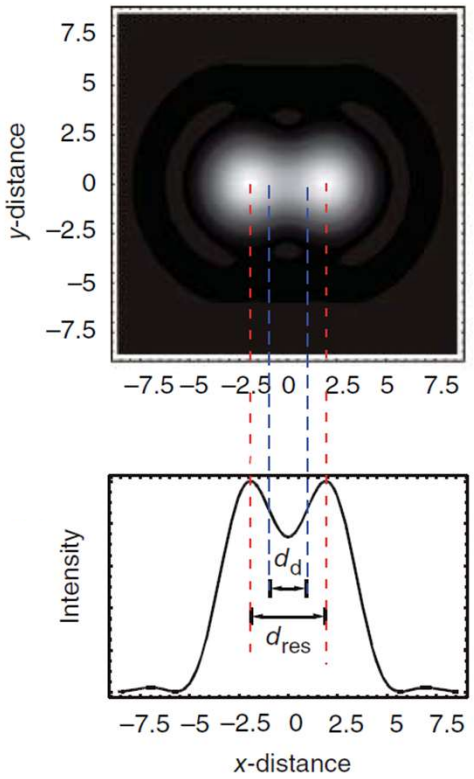

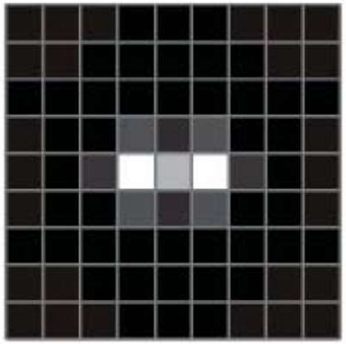

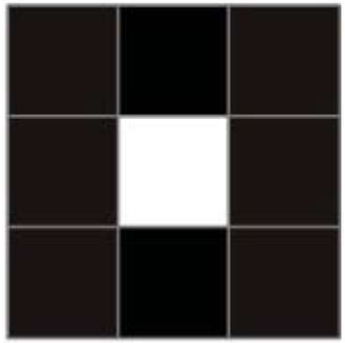

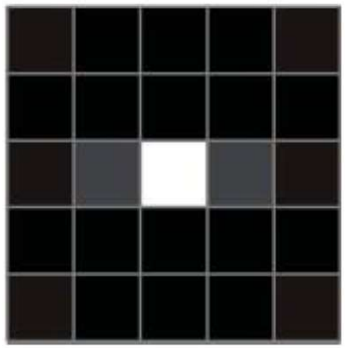

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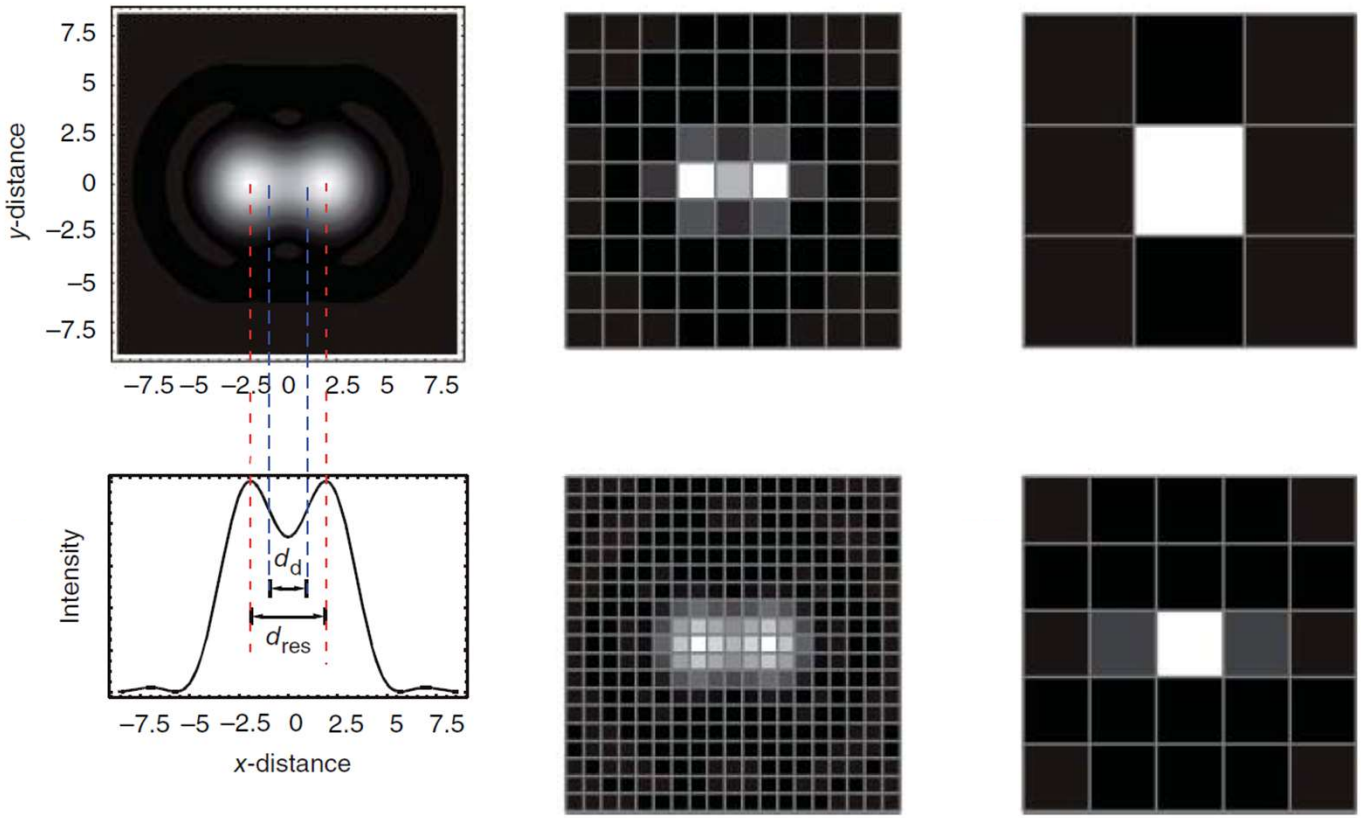

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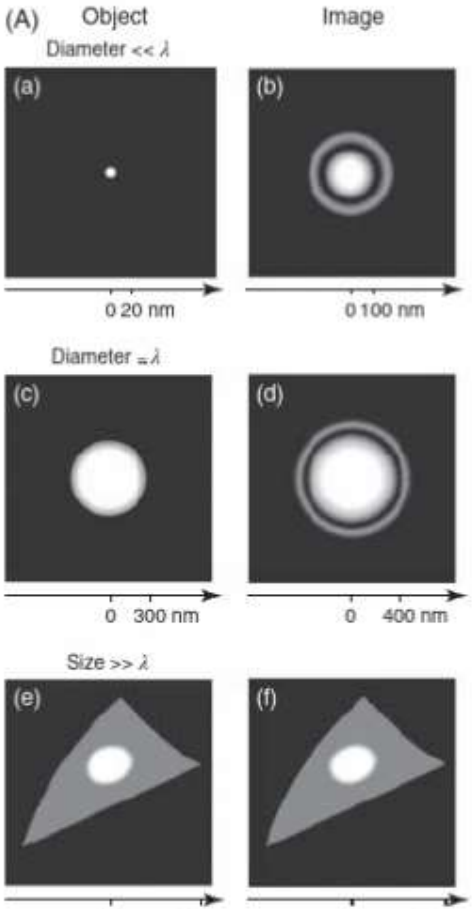
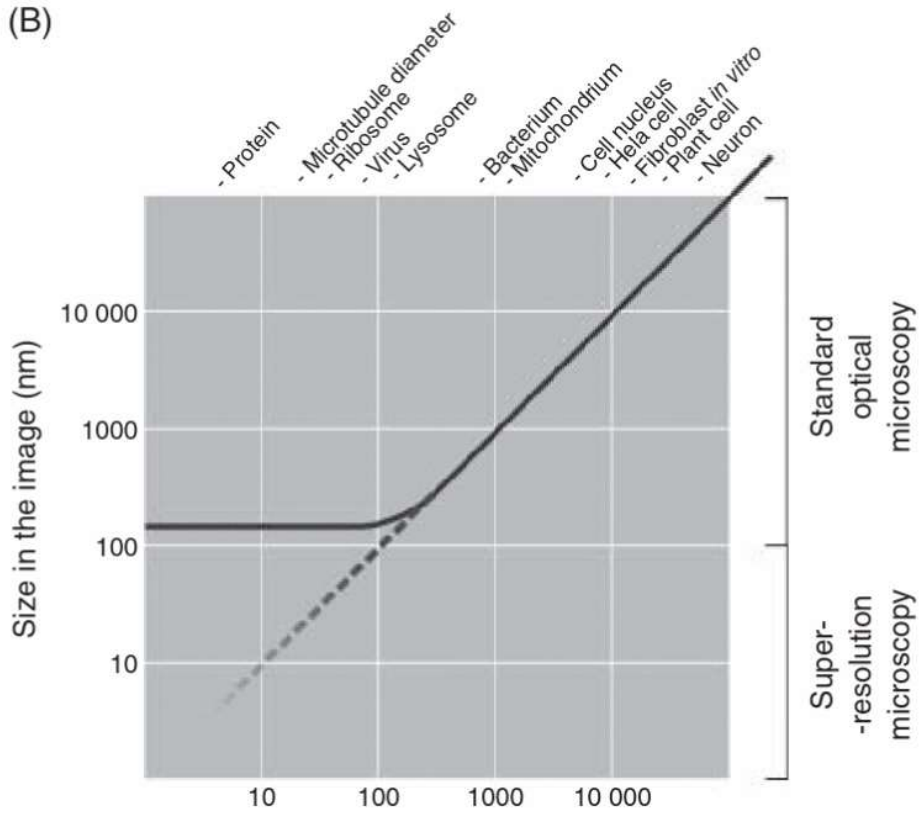
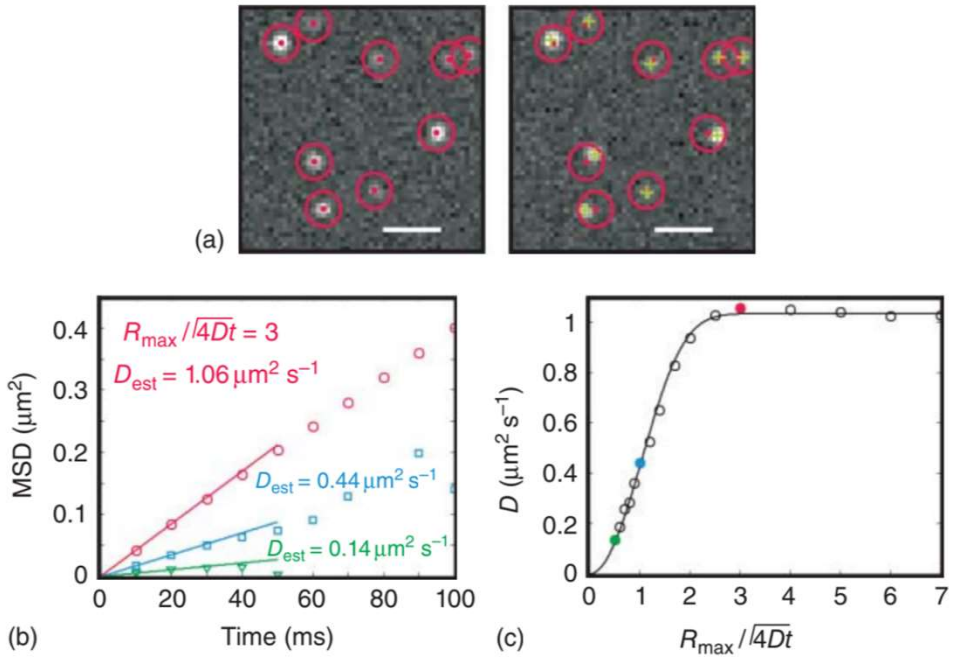
Moving from observation to quantification





10.1002/9783527687732

Quantifying single particle trajectories: symmetric single particles



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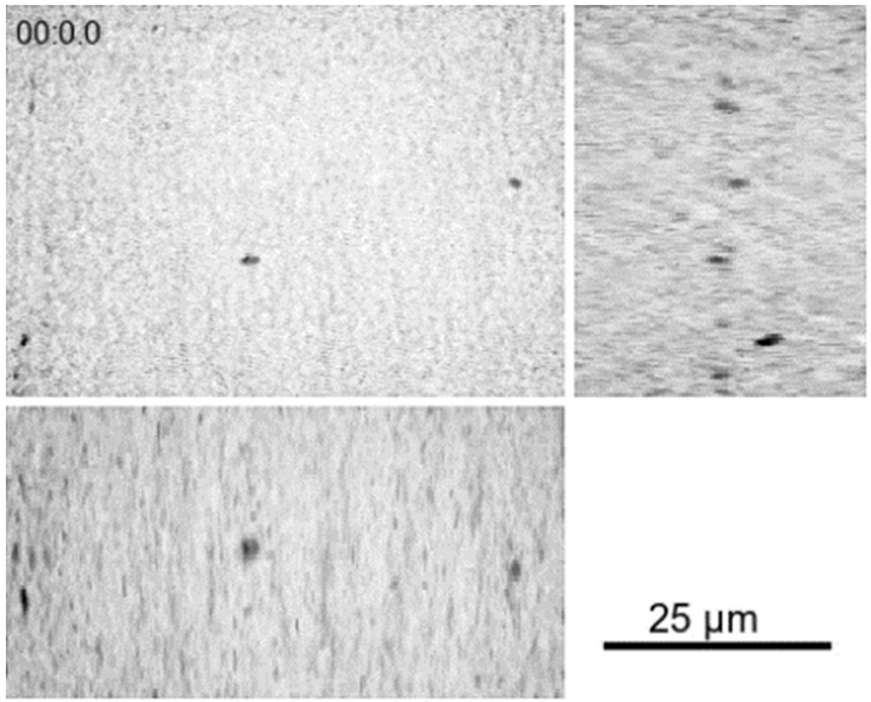
Quantifying single particle trajectories: large objects



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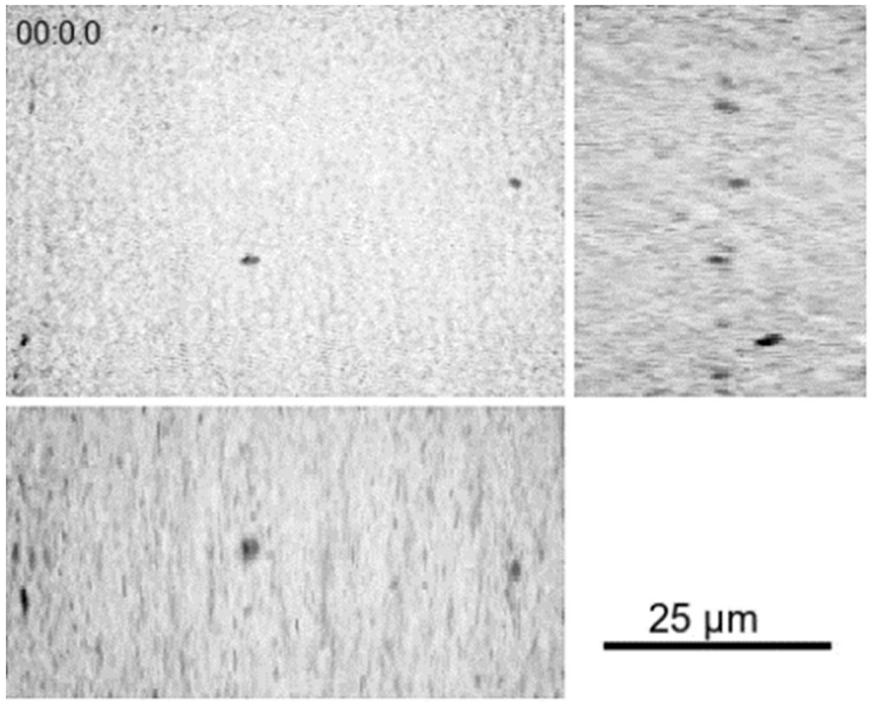
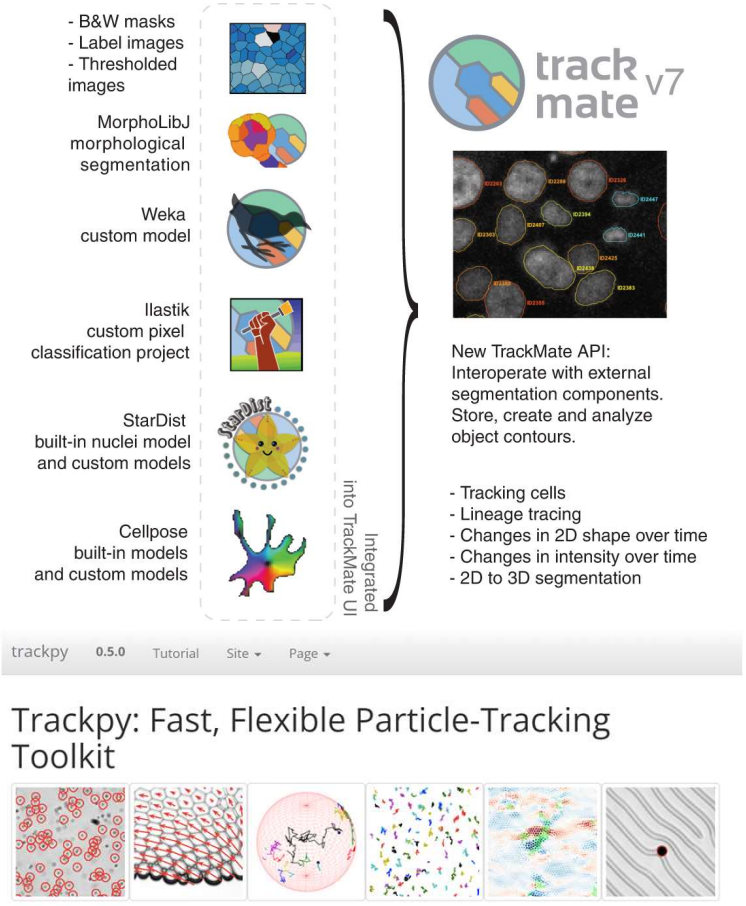
Quantifying single particle trajectories: large objects





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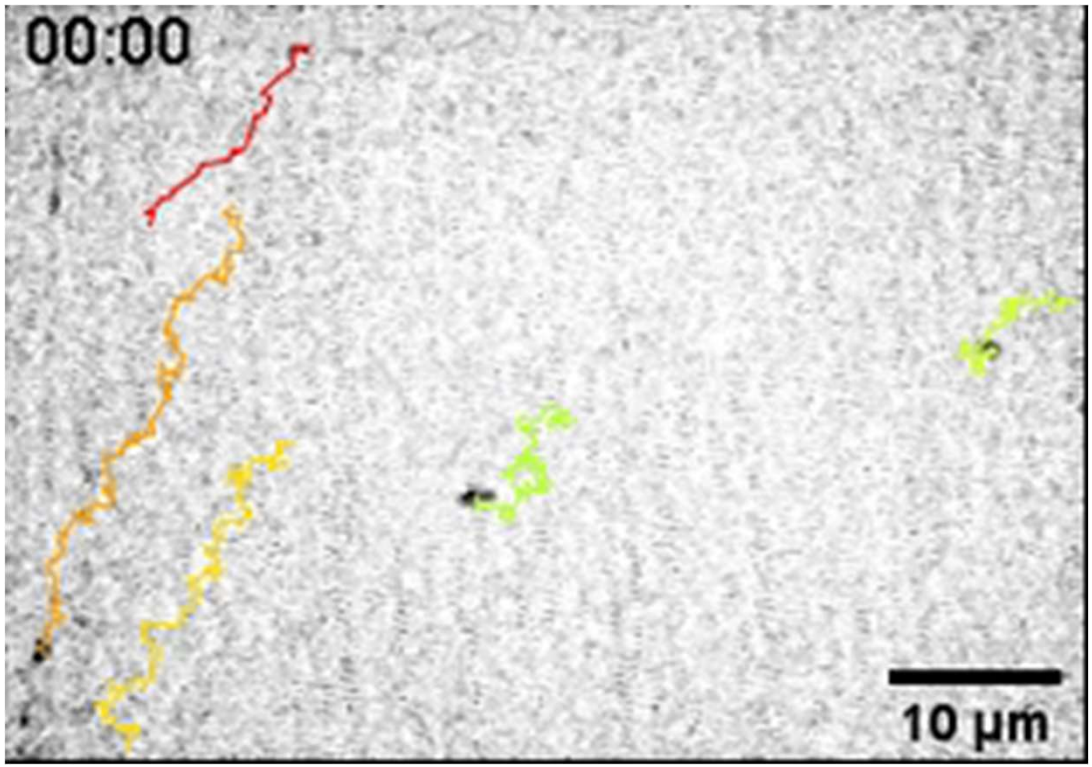

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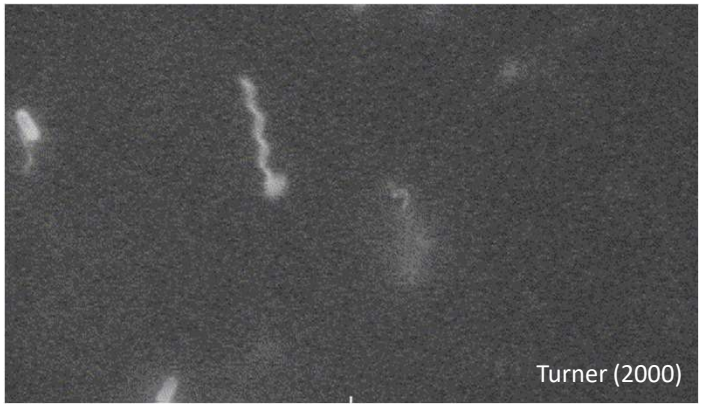
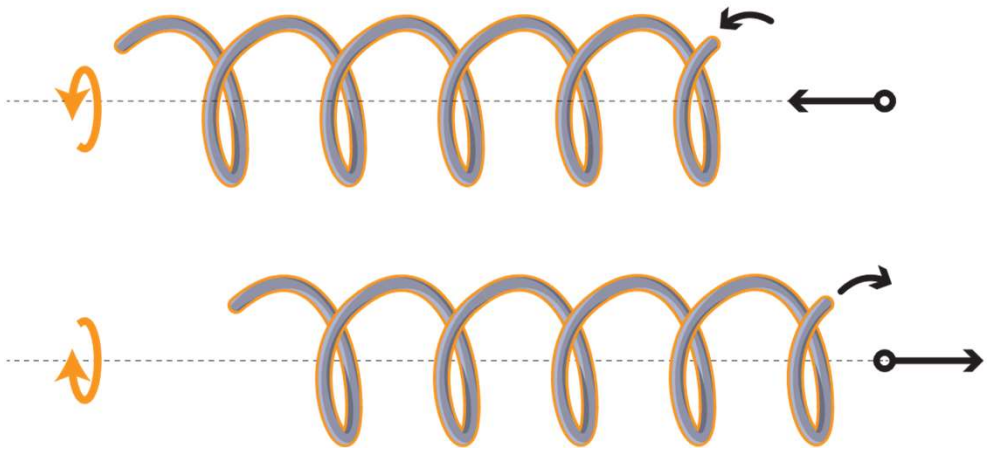
Quantifying single particle trajectories: asymmetric single particles





10.7554/eLife.57681

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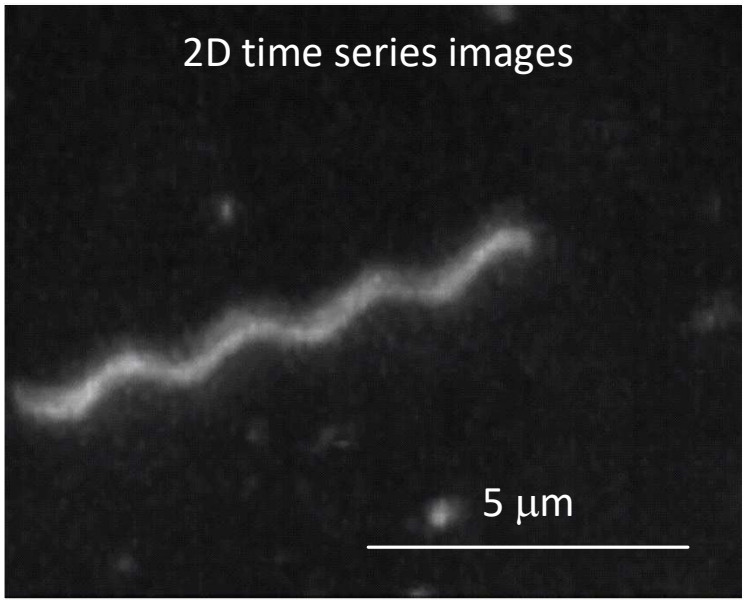
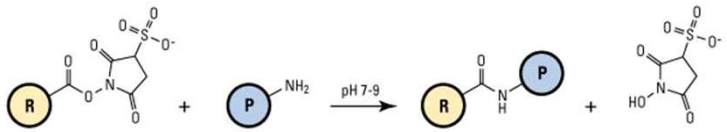
Helix particles have multiple diffusion coefficients





Thermo Fisher

rotational diffusion

$$A = \frac{D_\psi}{D_\parallel D_\psi - D_{\parallel\psi}^2} k_B T$$

translational diffusion

co-diffusion

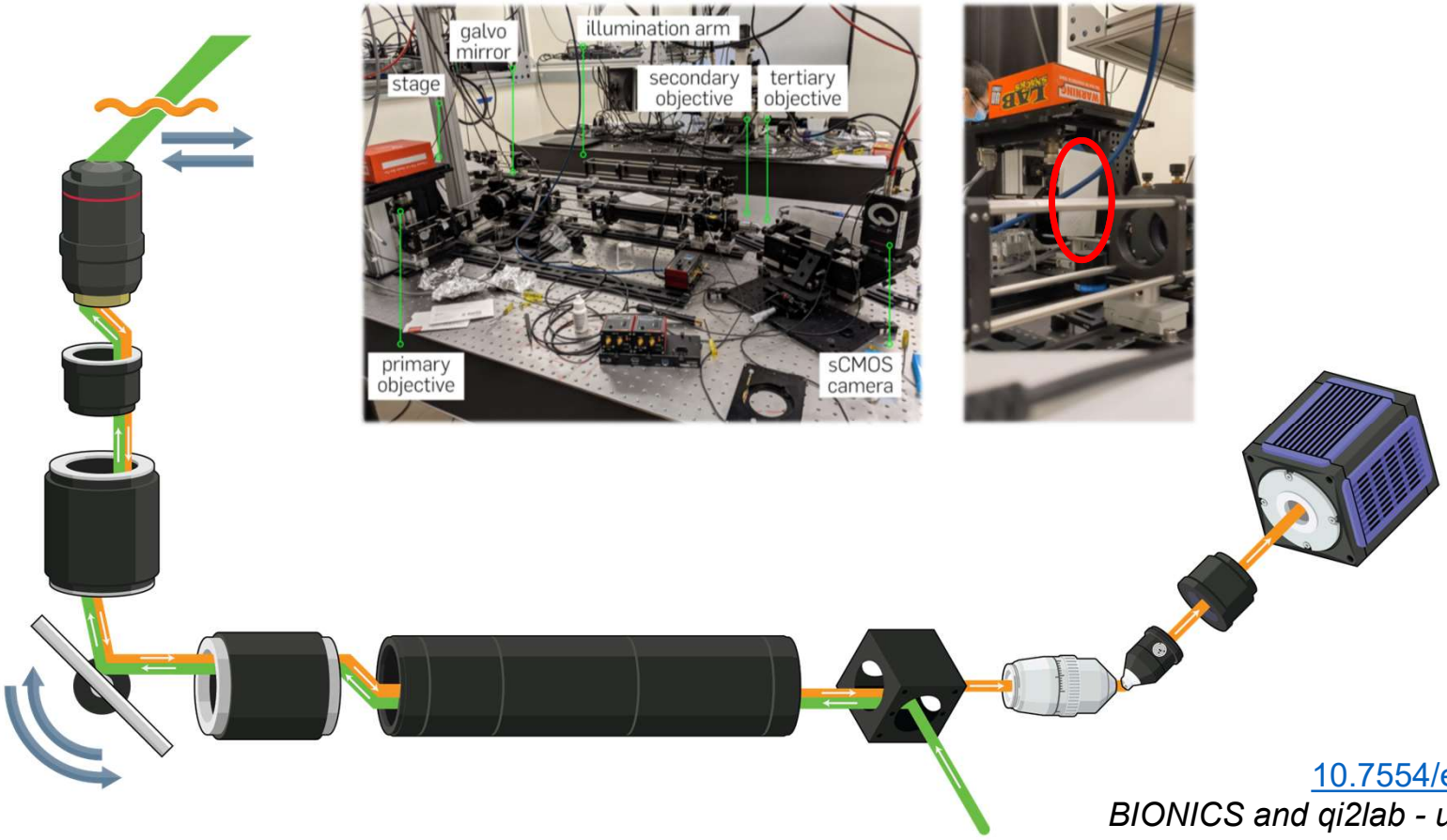
$$B = \frac{-D_{\parallel\psi}}{D_\parallel D_\psi - D_{\parallel\psi}^2} k_B T$$

$$D = \frac{D_\parallel}{D_\parallel D_\psi - D_{\parallel\psi}^2} k_B T$$

10.7554/eLife.57681

and qi2lab - unpublished

Need to track flagellum in 3D over time



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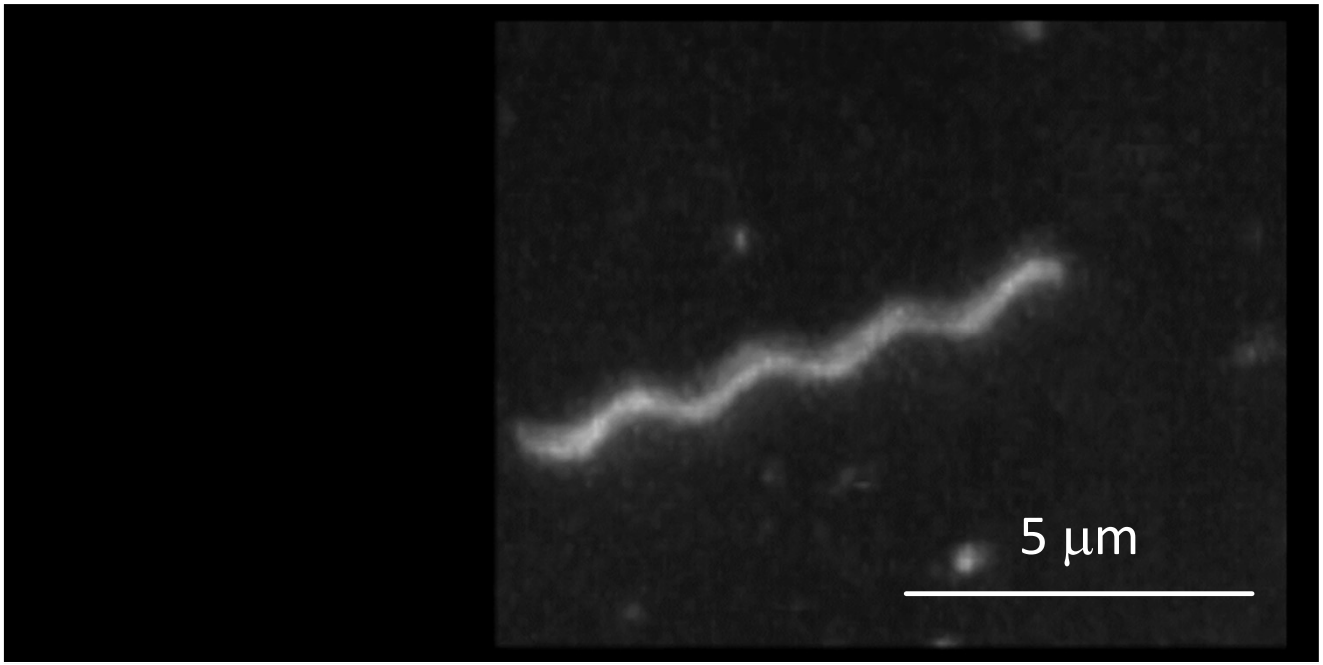
Need to track flagellum in 3D over time without changing inertia of fluid



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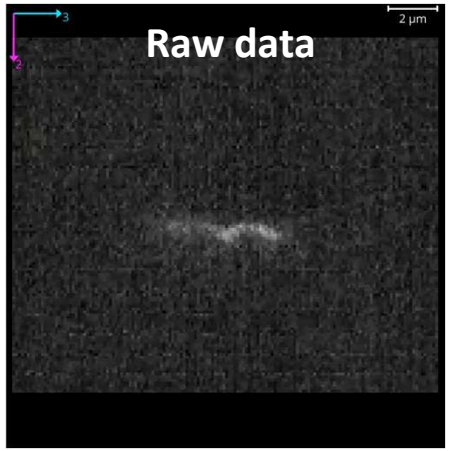
3D + time tracking

Segmentation of complex 3D shapes

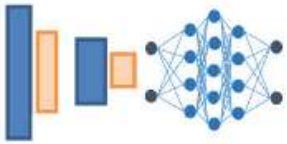
time domain

space domain

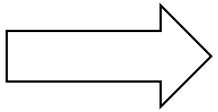


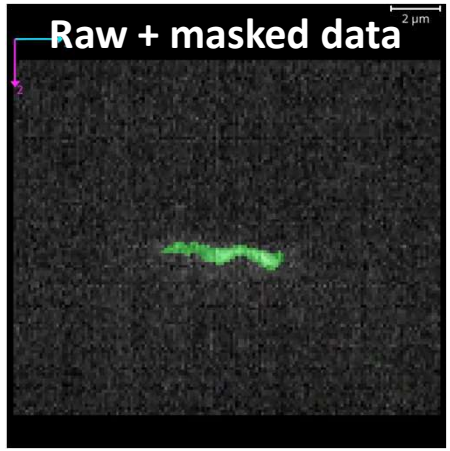
LabKit

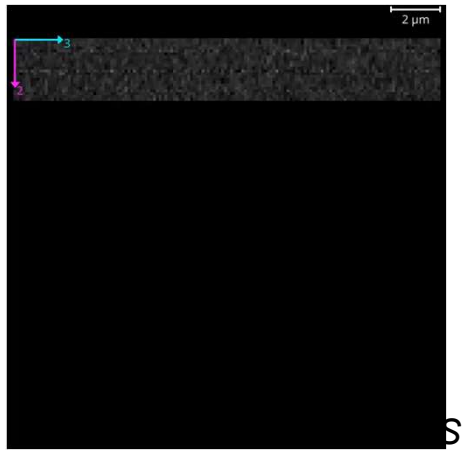
machine learning



Artz (2022)







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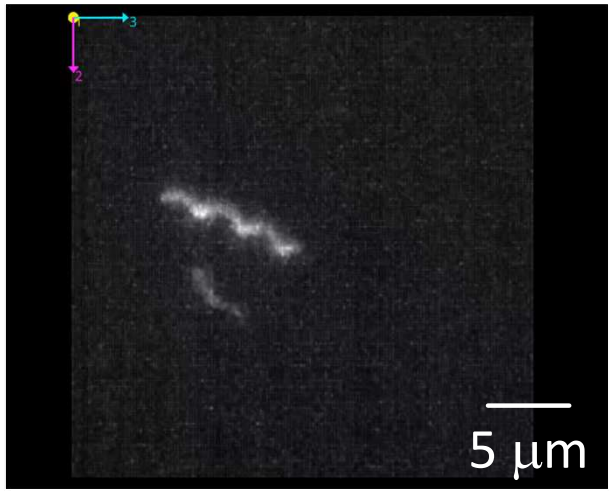
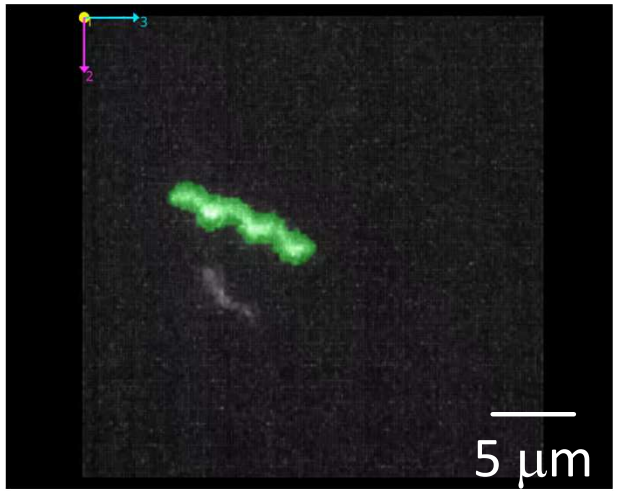
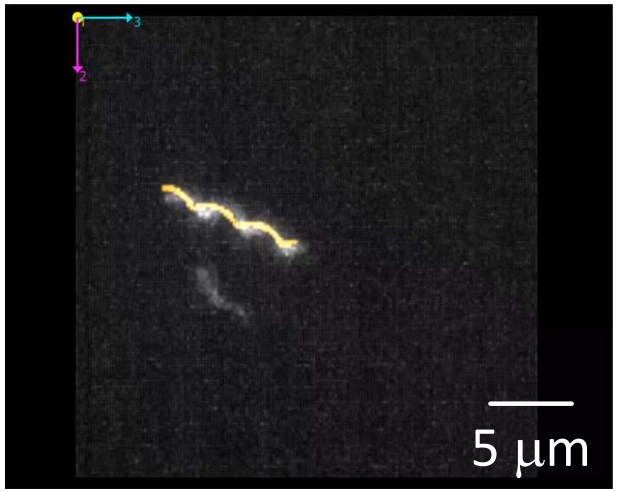
Full 3D plus time tracking of complex single particles



raw image



segmentation



tracking

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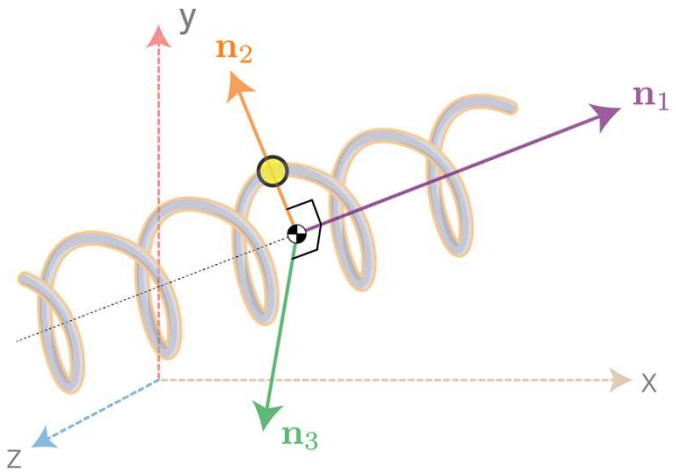
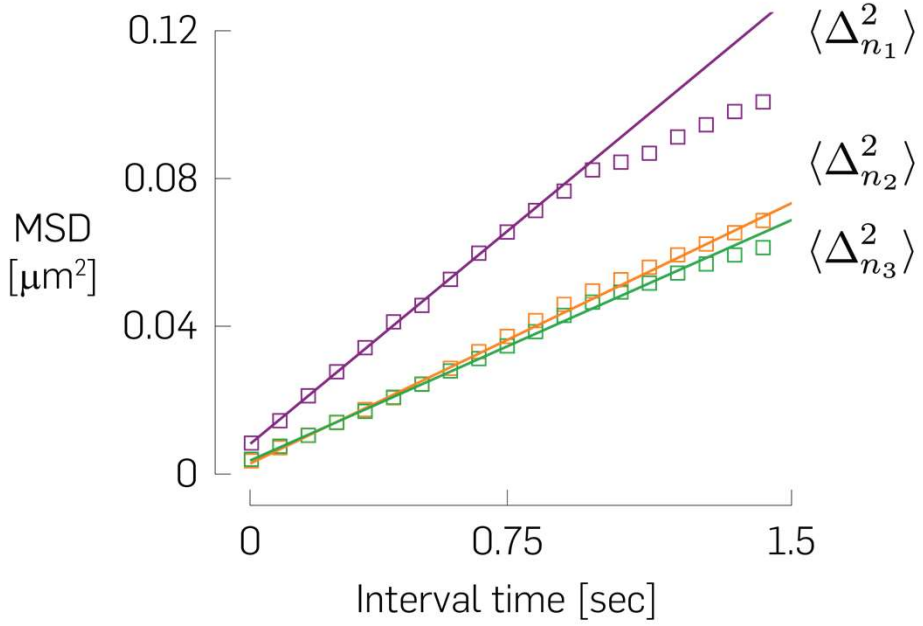
Extracting diffusion coefficients from flagellum tracks

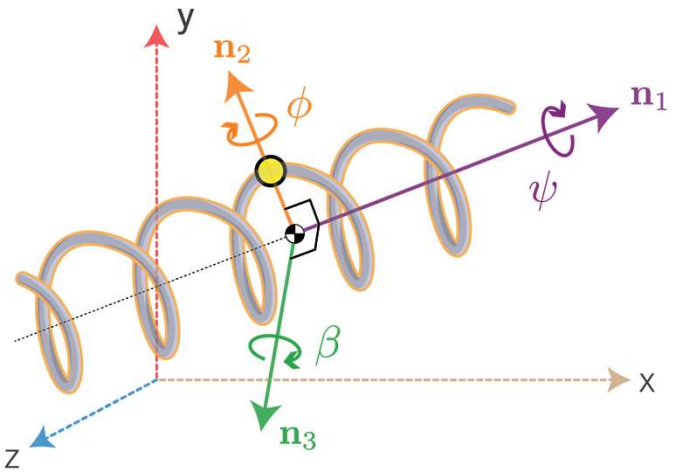


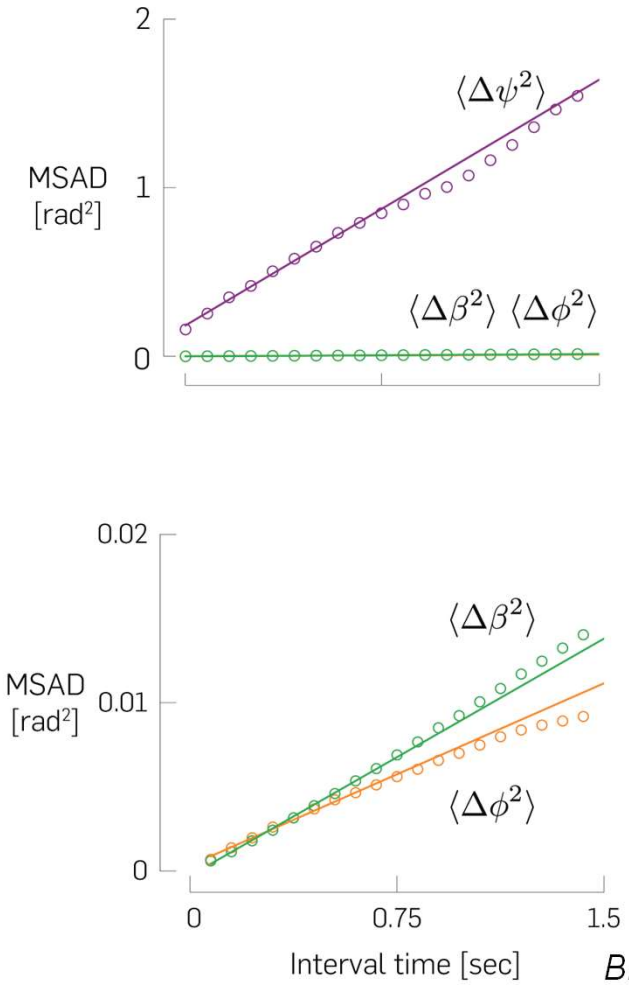


10.7554/eLife.57681

BIONICS and qi2lab - unpublished

Extracting diffusion coefficients from flagellum tracks





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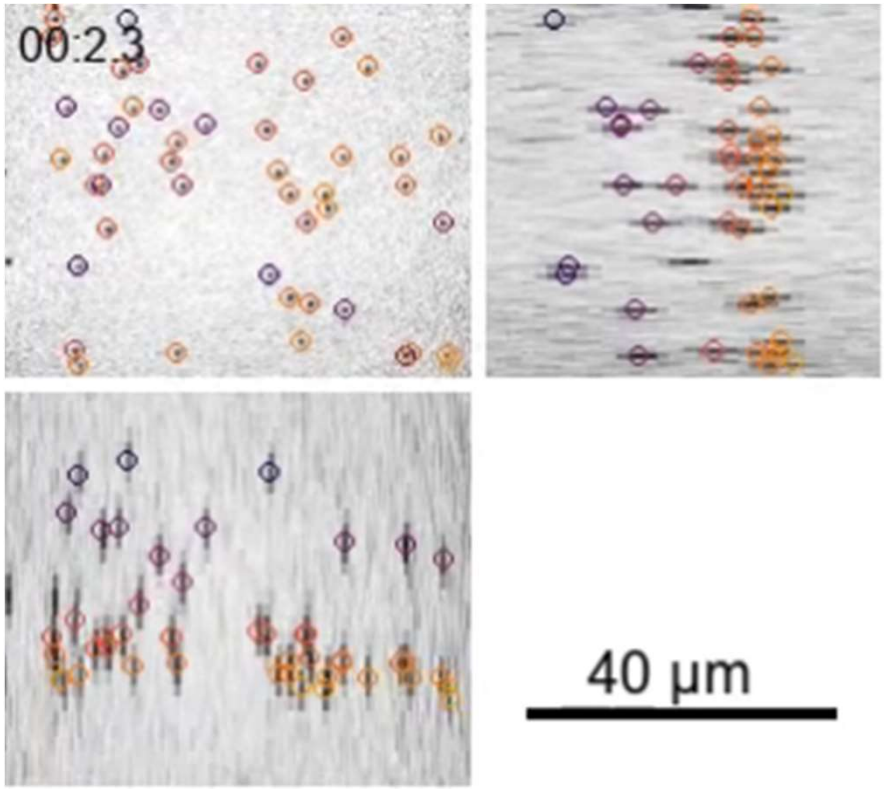
Summary



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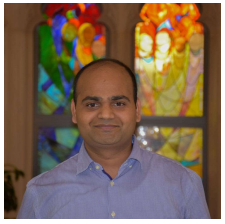

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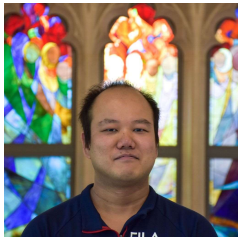

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Iterative FISH

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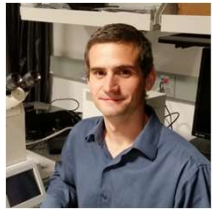

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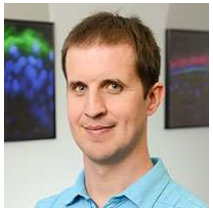

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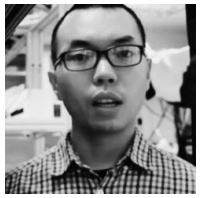

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Oblique plane microscopy