Single Particle Tracking

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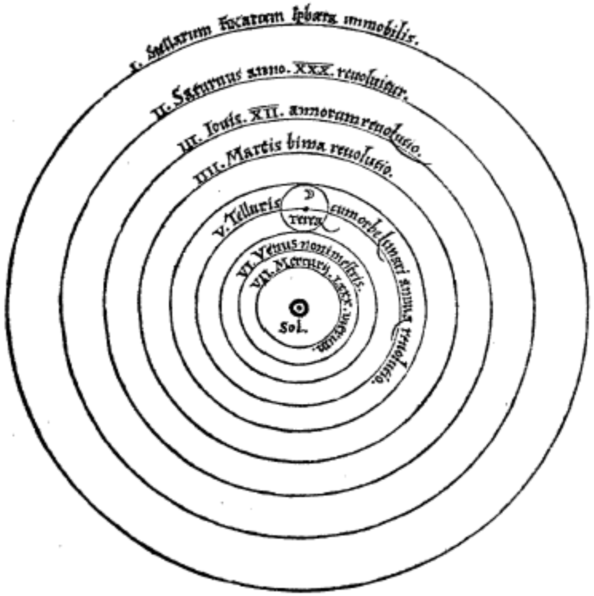
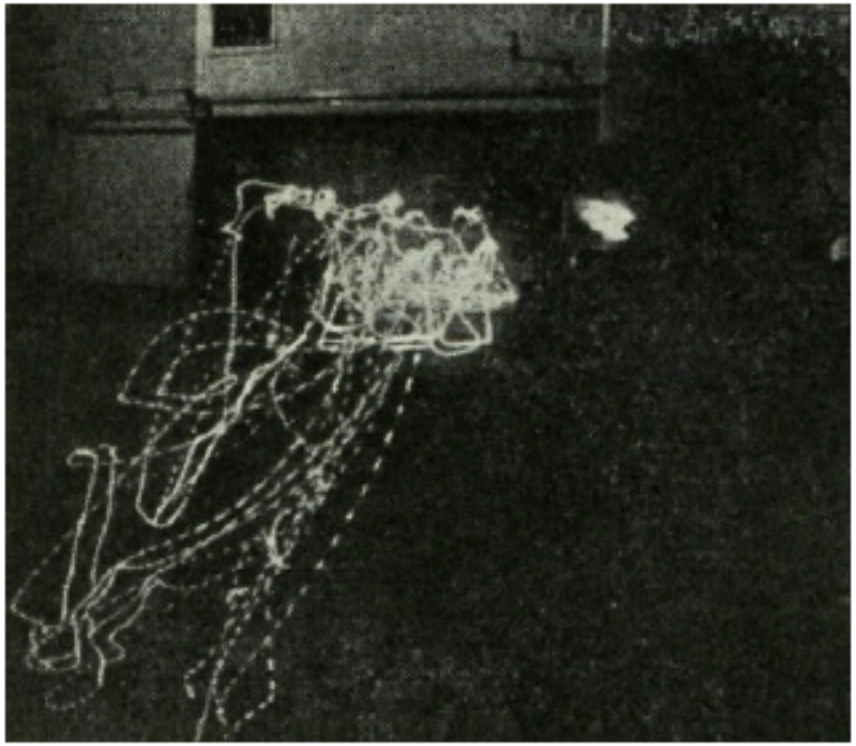
BIN LMU BioImaging Network Munich

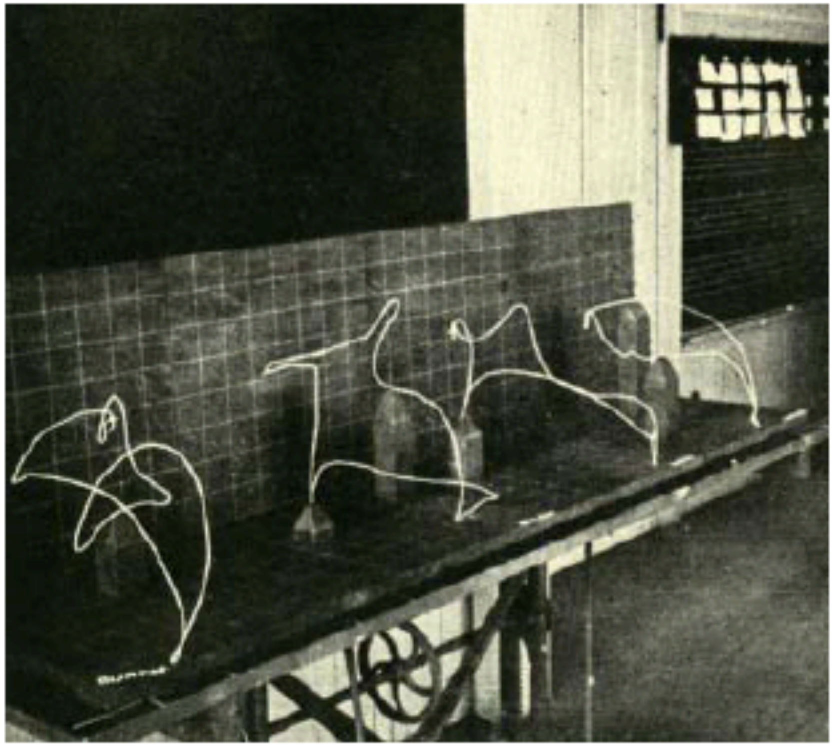
nsm nanosystems initiative munich CIPSM

Single Particle Tracking in 1500s



SPT in 1900s

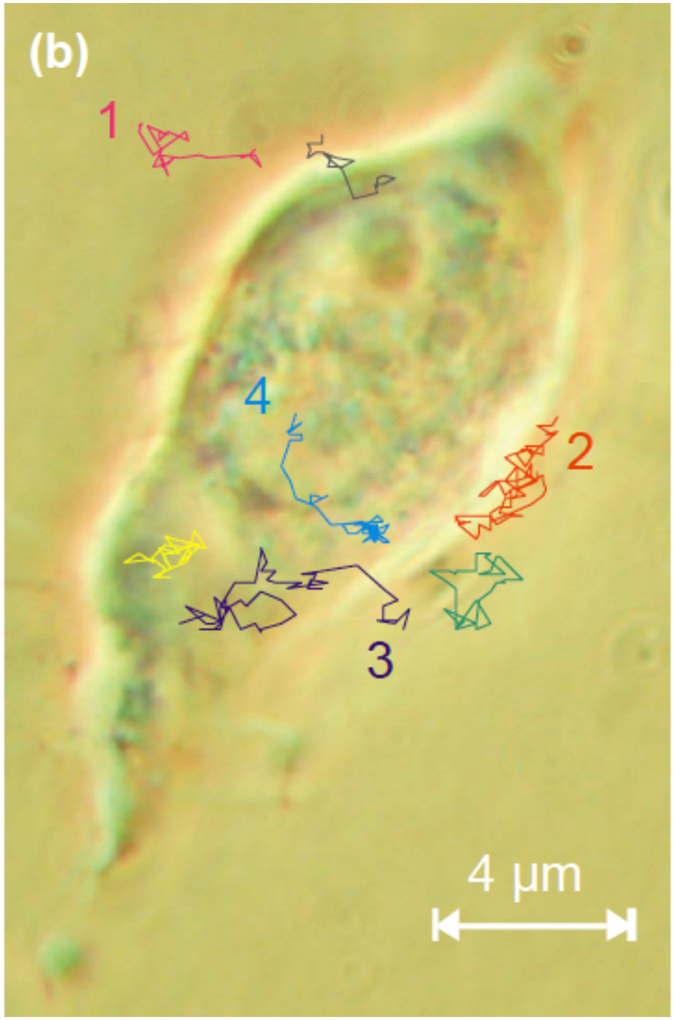




This is part of F. Gilberths work (1918): The efficiency of assembly line processing could be raised by understanding (and improving) the 3D trajectories of the workers movements.

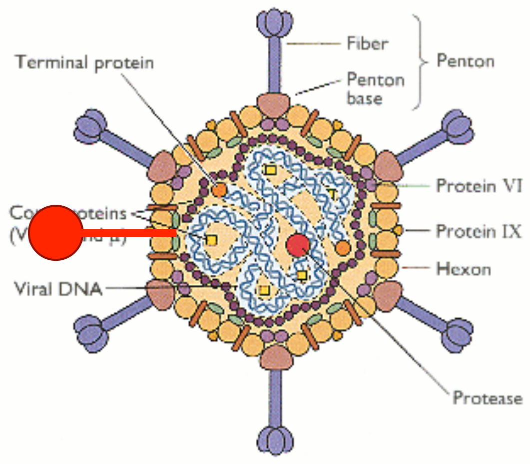
Single Particle Tracking in 1984

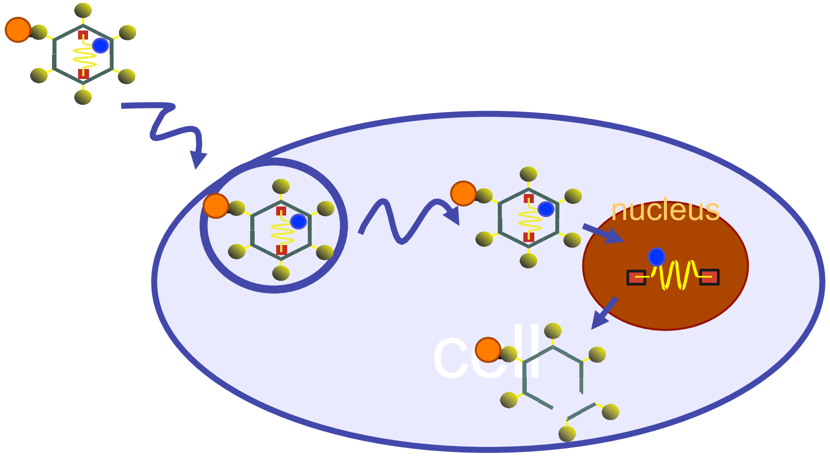
Lamb and Bräuchle 2007 *Physik J* **6**:39

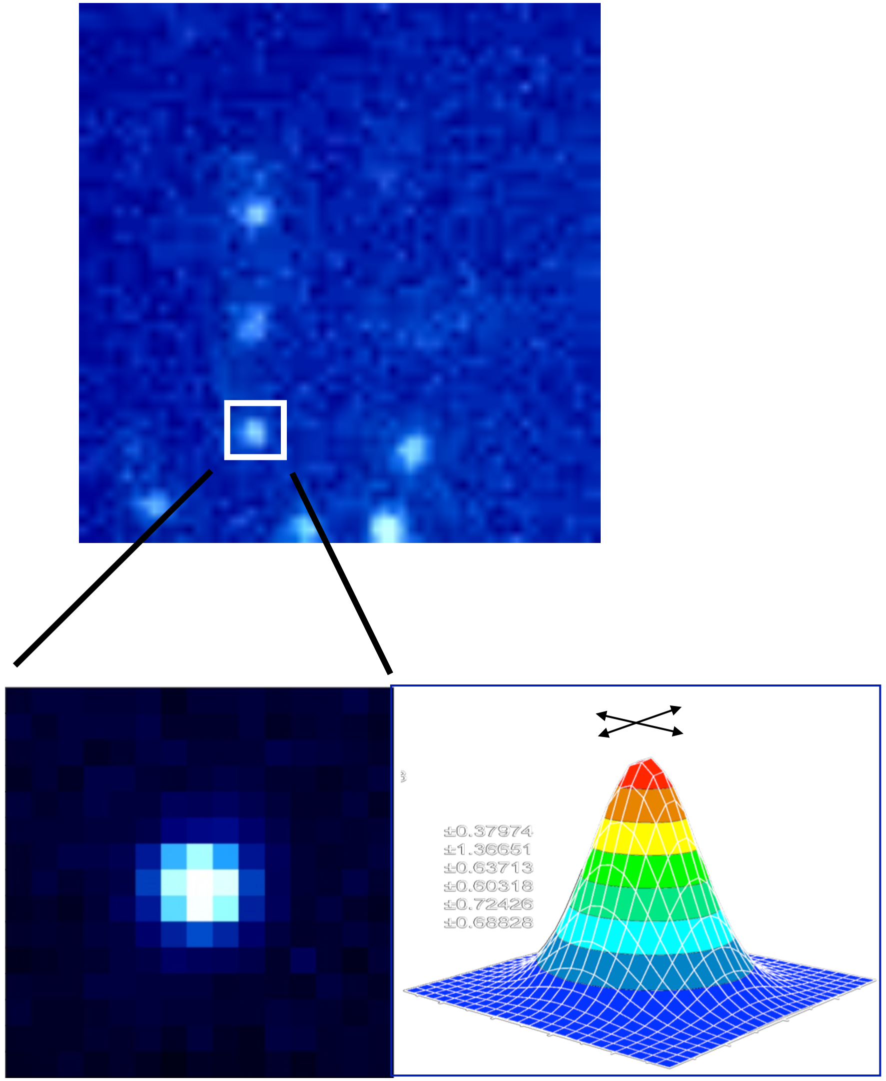


Seisenberger et al. *Science* 2001 ***294:*** 1929

Single Particle Tracking in 2D

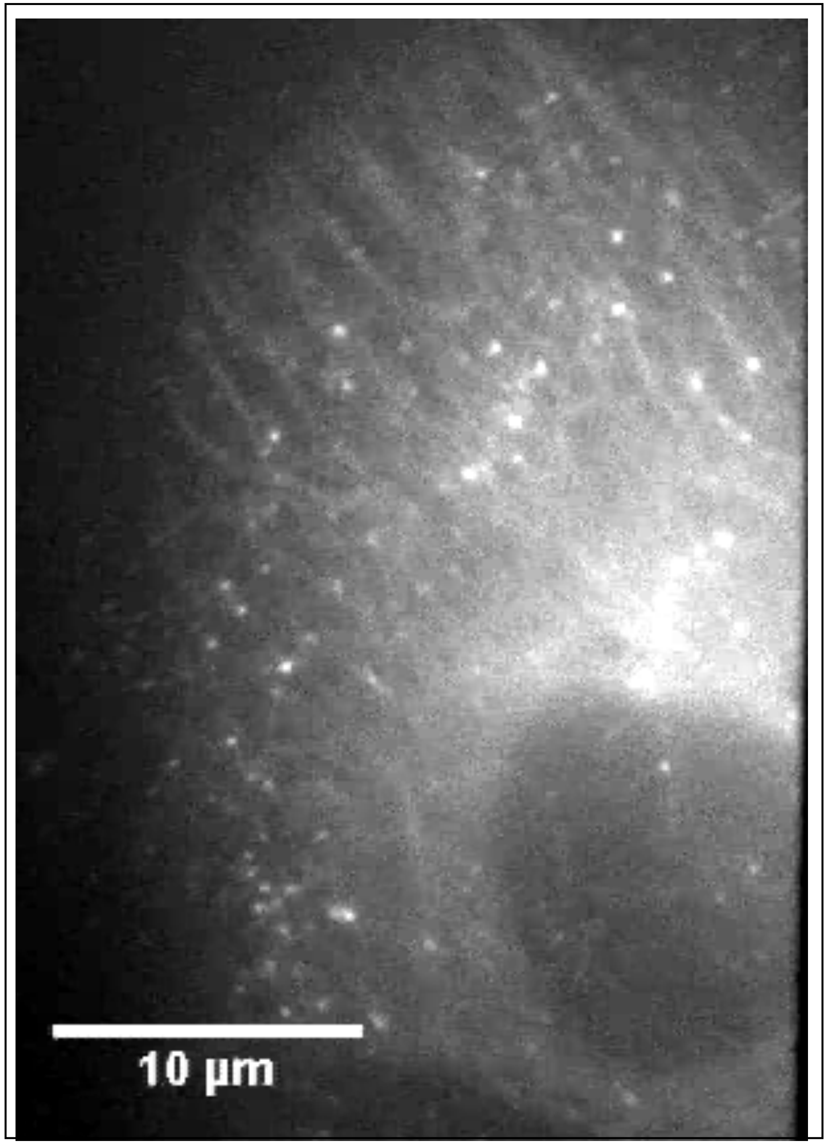




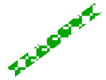


Science, 294 (2001)1929

Active PEI/DNA Polyplex Transport on Microtubules



Post injection: 30 min
Duration: 100 s
Resolution: 500 ms
Cell Type: HUH7

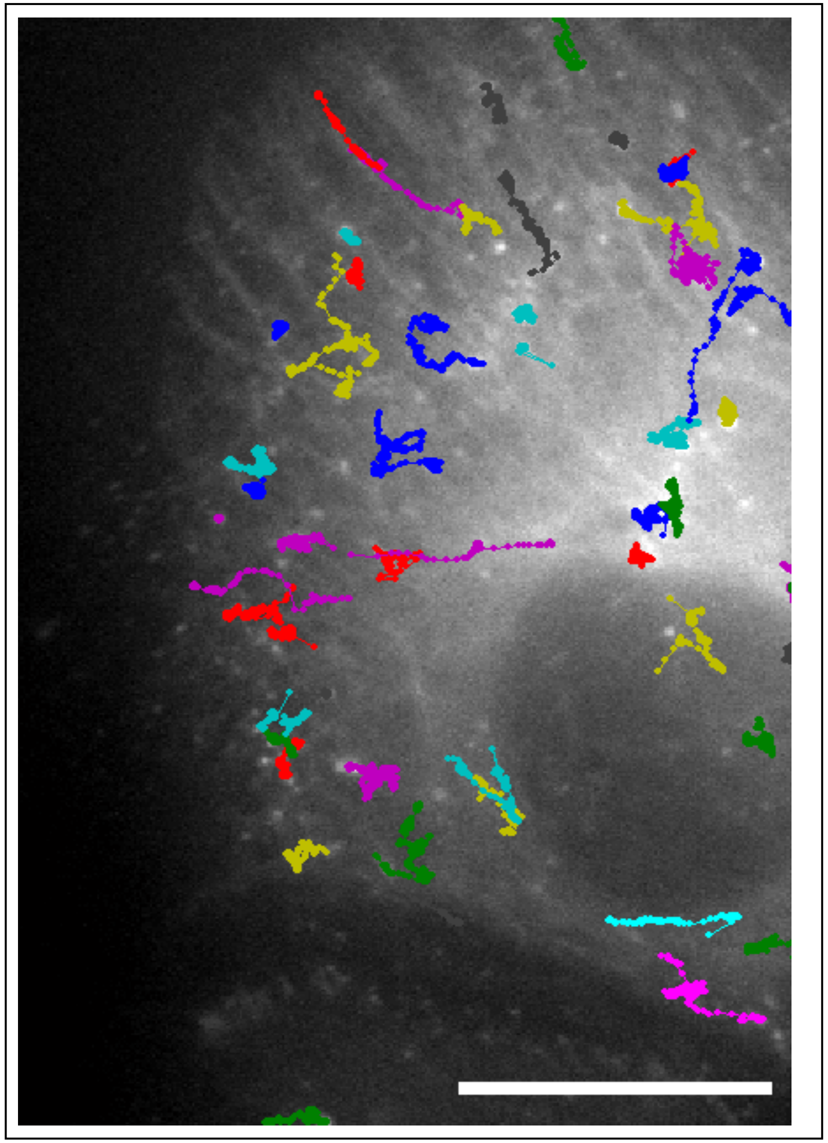

eGFP-labeled microtubules

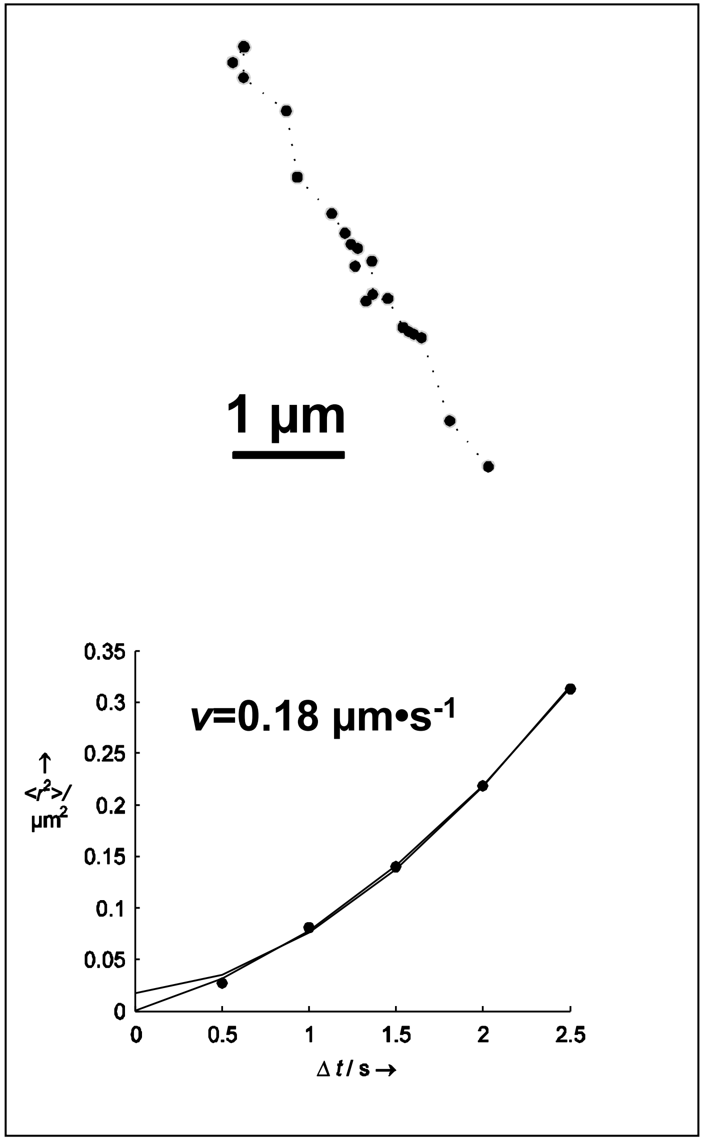

Cy3-labeled DNA/PEI particles

- Directed, active transport of particles;
- Polyplexes are transported along microtubules
- The direction of motion is random. There is no trend for the polyplexes to move towards the nucleus
- Block-and-pass events of nanoparticles observed

Bausinger et al., 2006
Angew. Chem. 45:1568

Active PEI/DNA Polyplex Transport on Microtubules



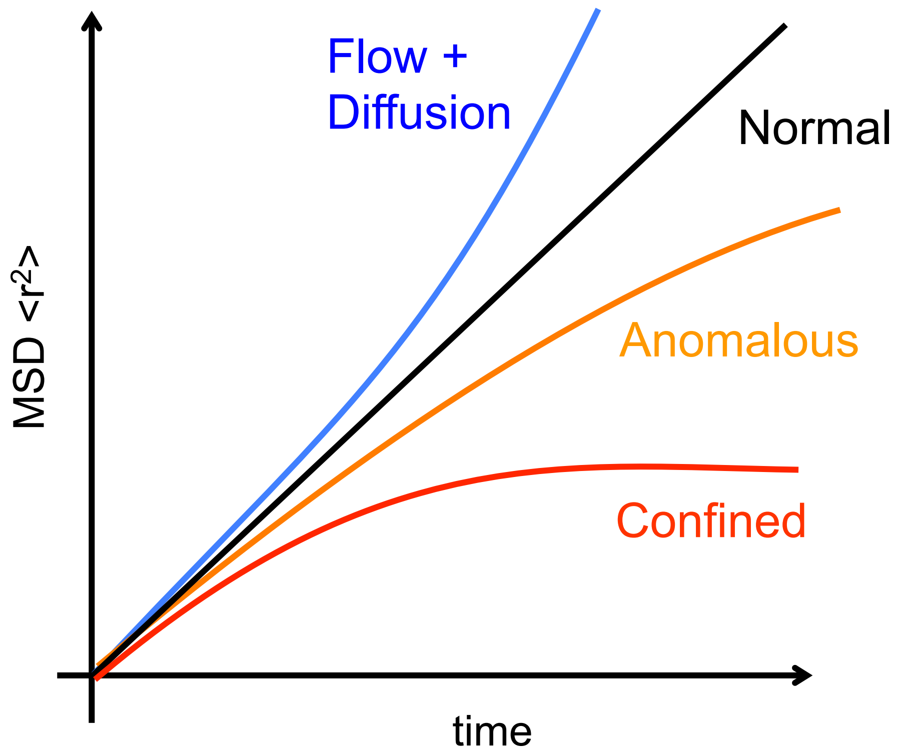


Types of Diffusion

The Diffusion Equation

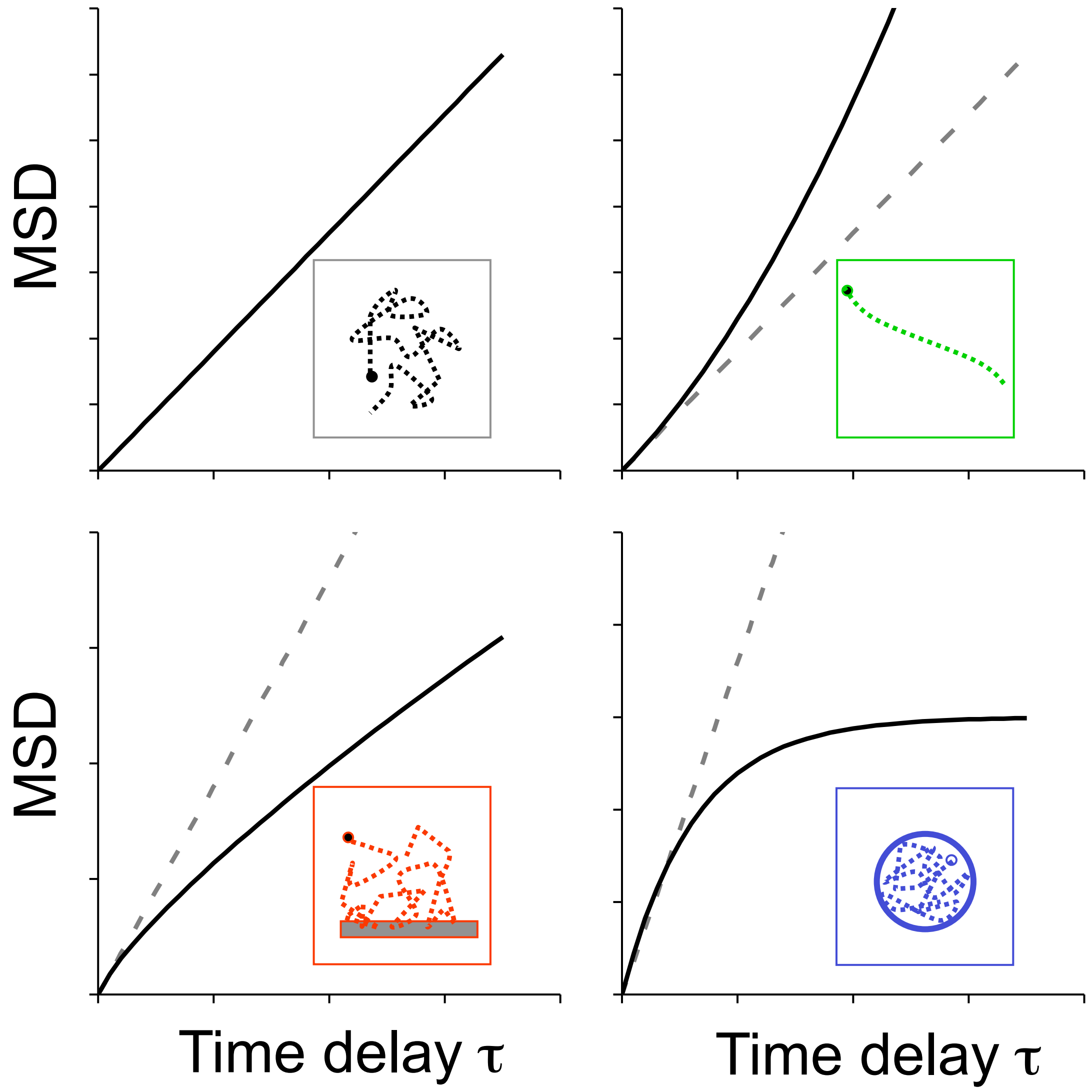
$$\frac{\partial C(\mathbf{r},t)}{\partial t} = D\nabla^2 C(\mathbf{r},t)$$

- Normal Diffusion (in 2D)
  - $\langle r^2 \rangle = 4\,D\,\tau$
- Diffusion with Flow
  - $\langle r^2 \rangle = 4\,D\,\tau + (V\tau)^2$
- Anomalous Diffusion
  - $\langle r^2 \rangle = 4\,D\,\tau^{\alpha}$
- Corralled Diffusion
  - $\langle r^2 \rangle = \langle r_C^2 \rangle \cdot \left[1 - A_1 \exp\left(-4A_2 D\tau / \langle r_C^2 \rangle\right)\right]$



Mean-Squared-Displacement

$$\mathrm{MSD}(\tau)=\left\langle r^2(\tau)\right\rangle=\left\langle\left[\mathrm{x}(\mathrm{t})-\mathrm{x}(\mathrm{t}+\tau)\right]^2+\left[\mathrm{y}(\mathrm{t})-\mathrm{y}(\mathrm{t}+\tau)\right]^2\right\rangle_t$$



The 'shape' of the trajectory is quantified through the MSD vs τ relationship

Accuracy of SPT

$$\left\langle (\Delta x)^2 \right\rangle = \frac{s^2}{N} + \frac{a^2/12}{N} + \frac{8\pi s^4 B^2}{a^2 N^2}$$

Δx = error in the particle position

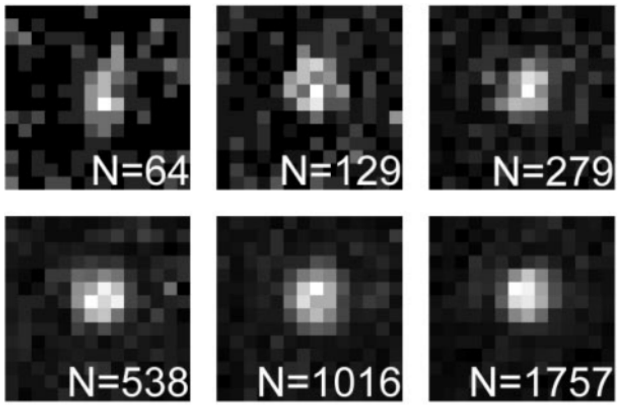
s = standard deviation of the PSF

N= number of photons detected

a = pixel size

B = background noise

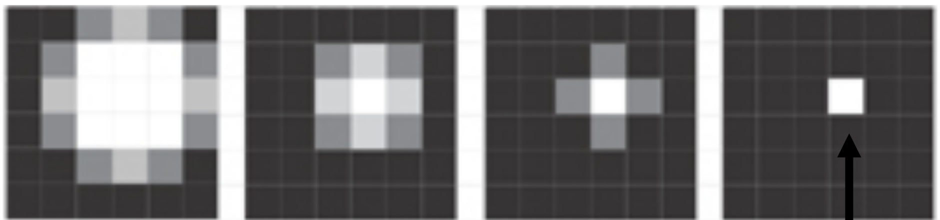
photon noise



background noise

B increases

pixelization noise



a increases

a > diffraction limit

Thompson et al. *Biophys J* (2002)

Accuracy in SPT

$$\left\langle (\Delta x)^2 \right\rangle = \frac{s^2}{N} + \frac{a^2/12}{N} + \frac{8\pi s^4 B^2}{a^2 N^2}$$

photon noise

pixelization noise

background noise

Δx = error in the particle position

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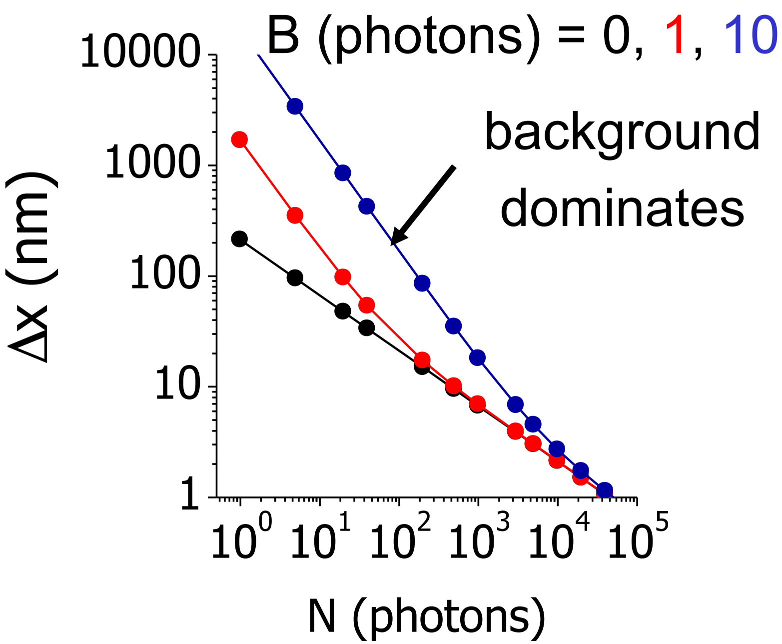
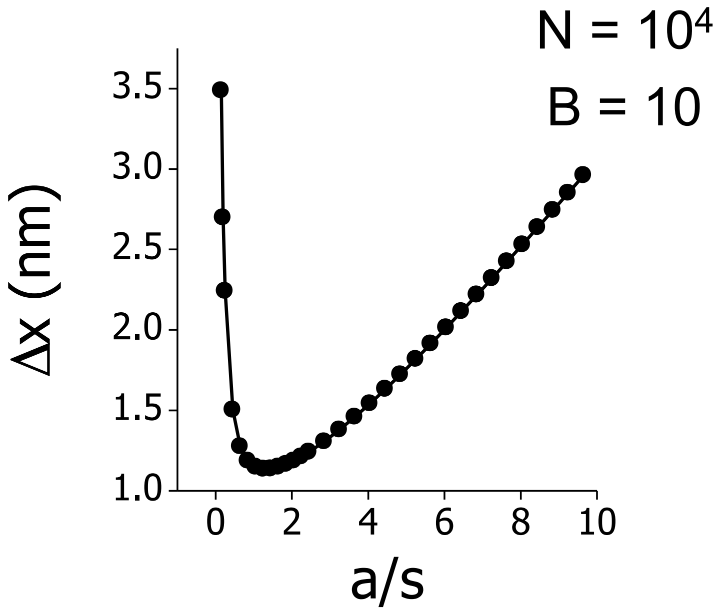


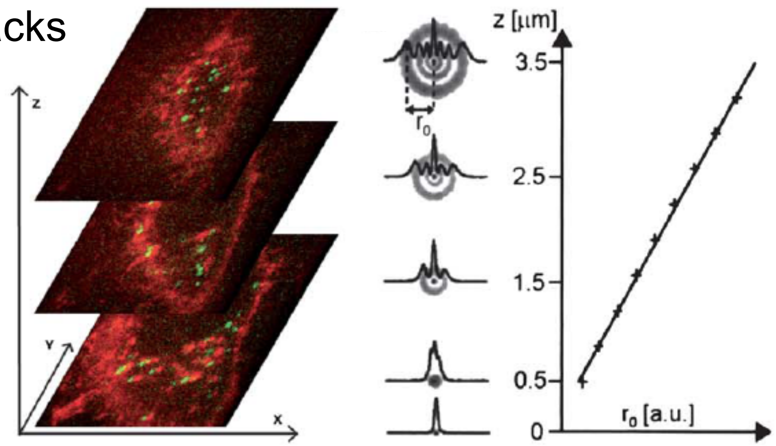


Particle Tracking

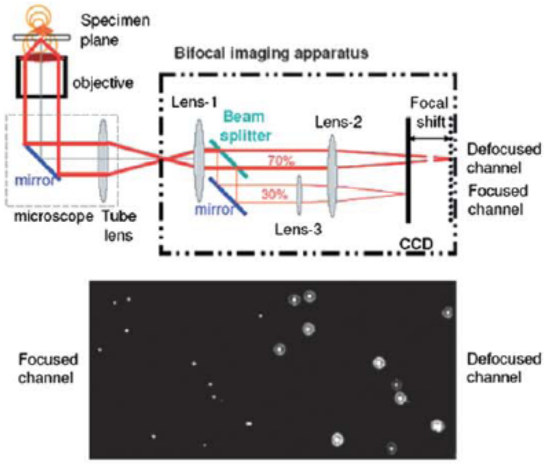
June 30th 1966, Wembley stadium

3D Tracking Methods

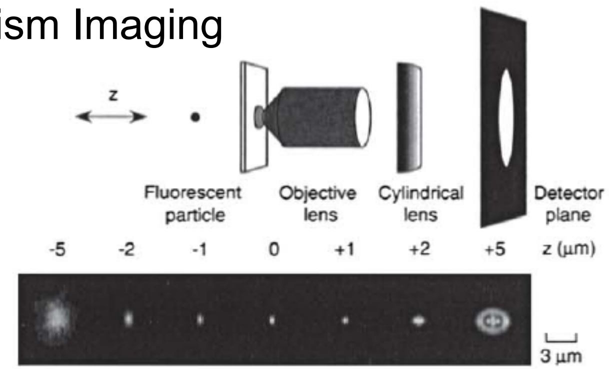
Z Stacks



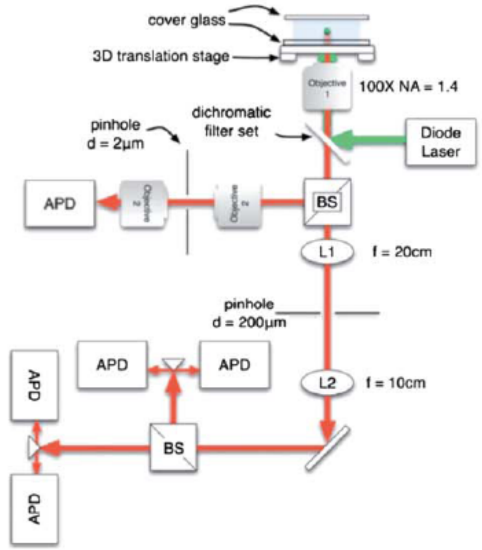
Bifocal Off Focus Imaging



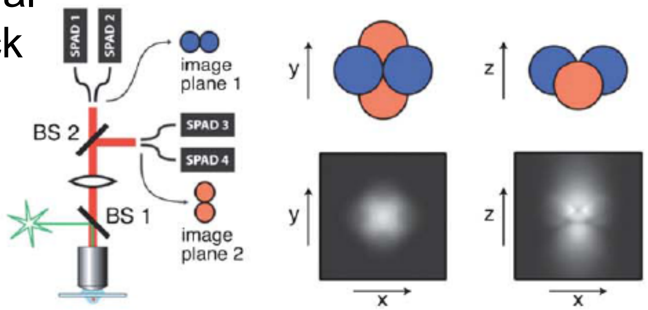
Astigmatism Imaging



Confocal Feedback Imaging



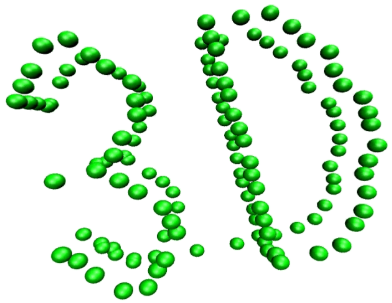
Tetrahedral Feedback



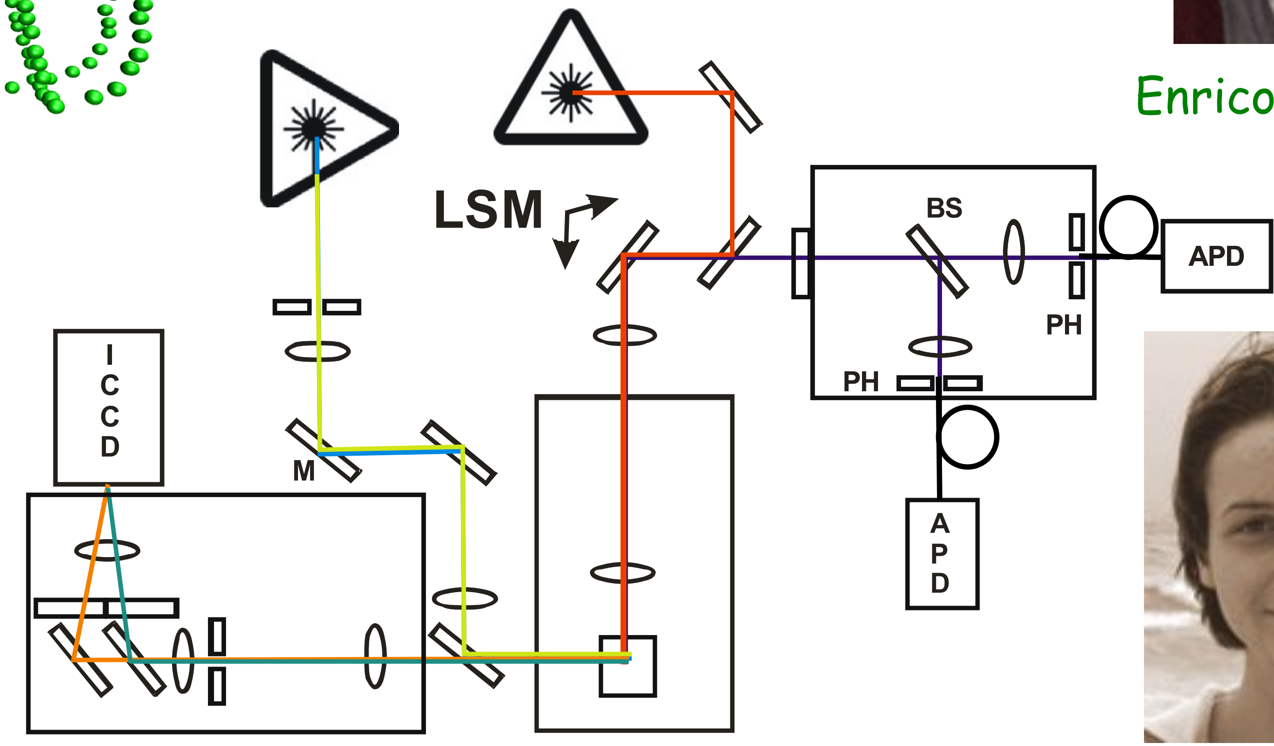
Dupont et al., *Nanoscale*, 2012

3-D Orbital Particle Tracking

with simultaneous bright field imaging



Enrico Gratton



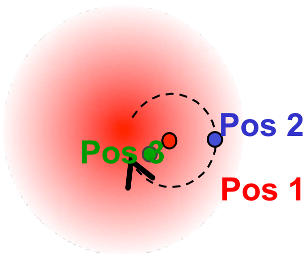
Fabian Wehnekamp

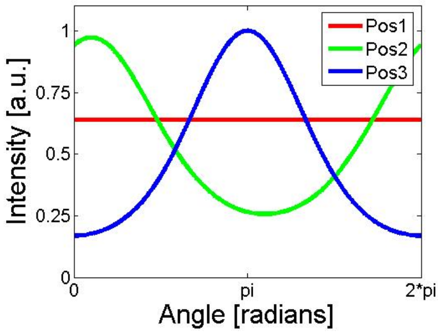
Bright Field

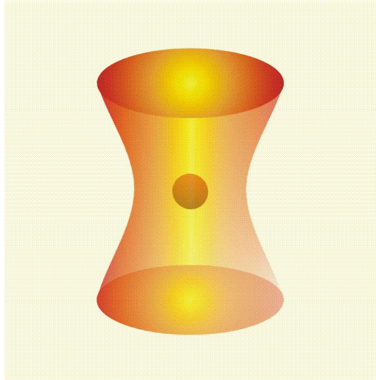
Dr. Aurelie Dupont

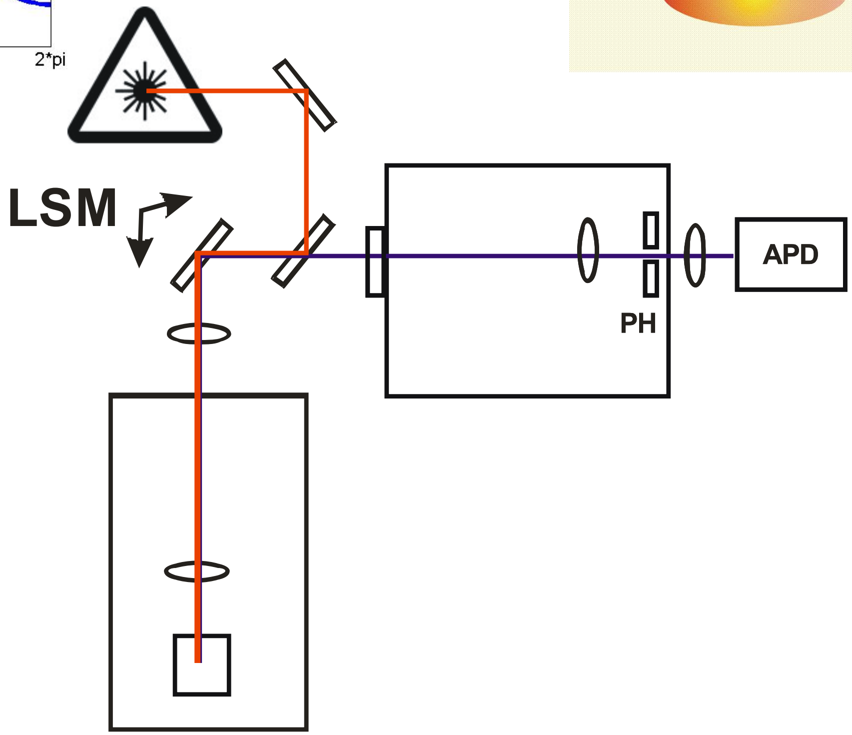
Orbital Particle Tracking

3D orbital tracking microscope (x,y)





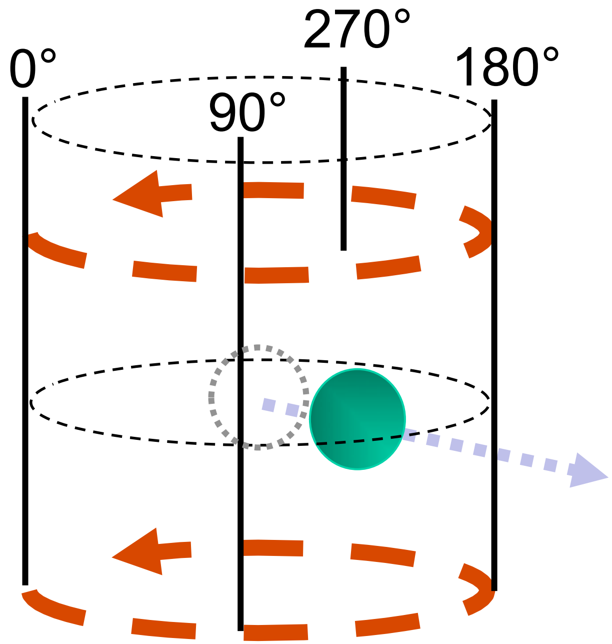


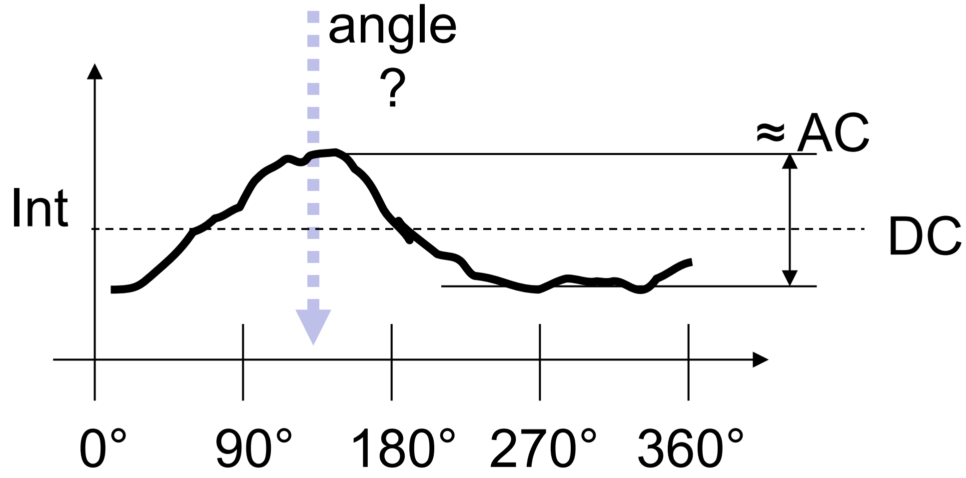


Estimate Position from Orbit

To locate the particle we need to know:

Angle, distance and height from center





FFT[$I(t)$] → DC and AC

$$DC \rightarrow < PSF(\vec{r} - \vec{r}_P) >$$

Average of the function along the orbit

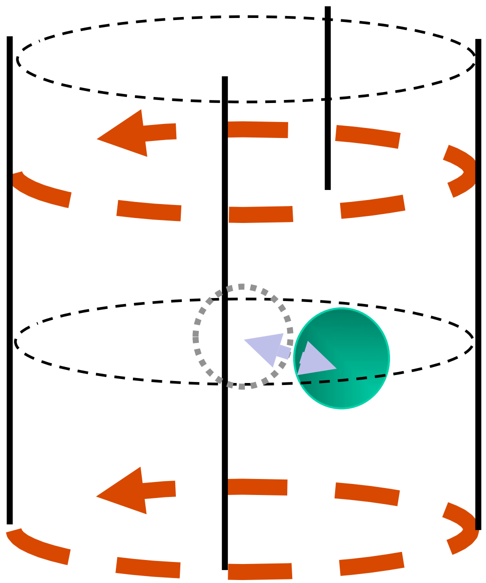
$$AC \rightarrow \Delta PSF(\vec{r} - \vec{r}_P)$$

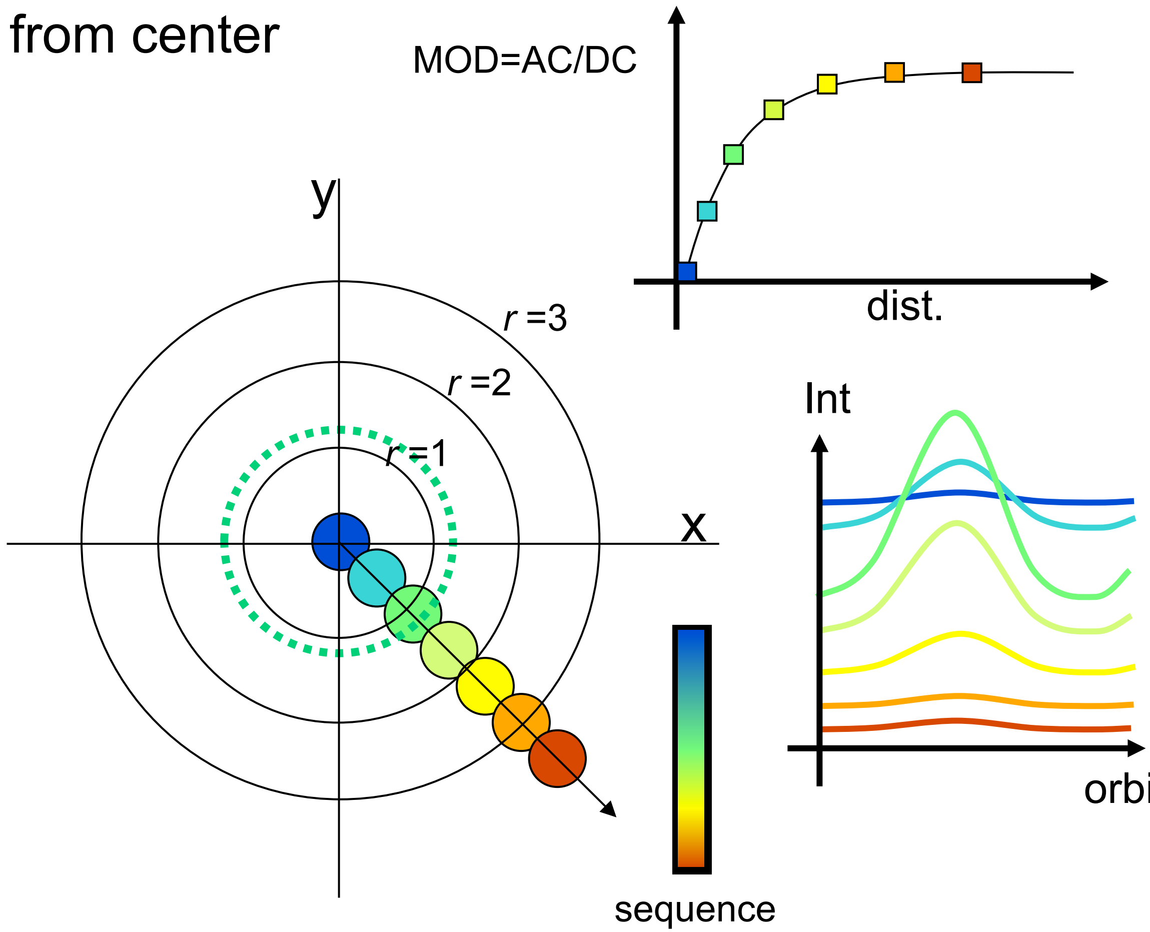
Variation of the function along the orbit

Estimate Position from Orbit

To locate the particle we need to know:

Angle, **distance** and height from center





$$DC \rightarrow < PSF(\vec{r} - \vec{r}_P) >$$

$$AC \rightarrow \Delta PSF(\vec{r} - \vec{r}_P)$$

Orbital Tracking: Mathematical Details

MICROSCOPY RESEARCH AND TECHNIQUE 63:34–49 (2004)

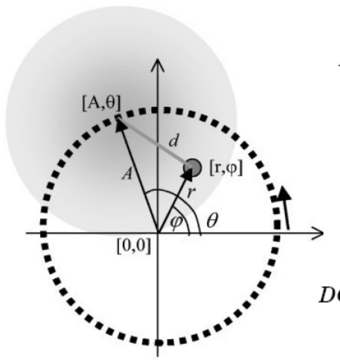
Distance Measurement by Circular Scanning of the Excitation Beam in the Two-Photon Microscope

KATARINA KIS-PETIKOVA AND ENRICO GRATTON
Laboratory of Fluorescence Dynamics, University of Illinois at Urbana-Champaign, Urbana, Illinois 61801-3080

Biophysical Journal Volume 88 April 2005 2919–2928

3-D Particle Tracking in a Two-Photon Microscope:

Valeria Levi, QiaoQiao Ruan, and Enrico Gratton
Laboratory for Fluorescence Dynamics



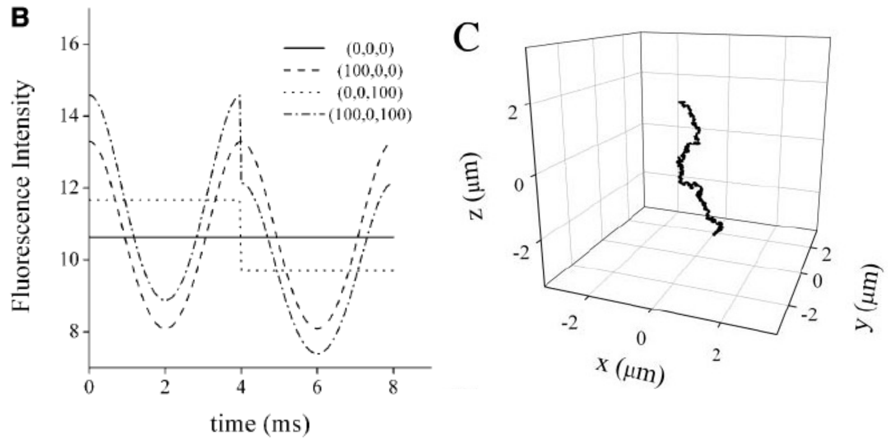
$$AC = b_1 = \frac{1}{\pi}\int_{-\pi}^{\pi} \cos\theta \cdot F_{01} \times \exp\left[-\frac{4}{w_0^2}(A^2 + r_1^2 - 2Ar_1\cos\theta)\right]d\theta = 2F_{01}\exp\left[-\frac{4}{w_0^2}(A^2 + r_1^2)\right]\cdot I_1\left(\frac{8Ar}{w_0^2}\right)$$

$$DC = a_0 = \frac{1}{2\pi}\int_{-\pi}^{\pi} F_{01}\exp\left[-\frac{4}{w_0^2}(A^2 + r_1^2 - 2Ar_1\cos\theta)\right] \times d\theta = F_{01}\exp\left[-\frac{4}{w_0^2}(A^2 + r_1^2)\right]\cdot I_0\left(\frac{8Ar}{w_0^2}\right) \quad (18)$$

$$\frac{\Delta d_{\min}}{w_0} = \frac{0.85}{\sqrt{F_{0m}}}$$

AC/DC:

$$MOD = 2 \cdot I_1\left(\frac{8Ar}{w_0^2}\right) \Big/ I_0\left(\frac{8Ar}{w_0^2}\right)$$

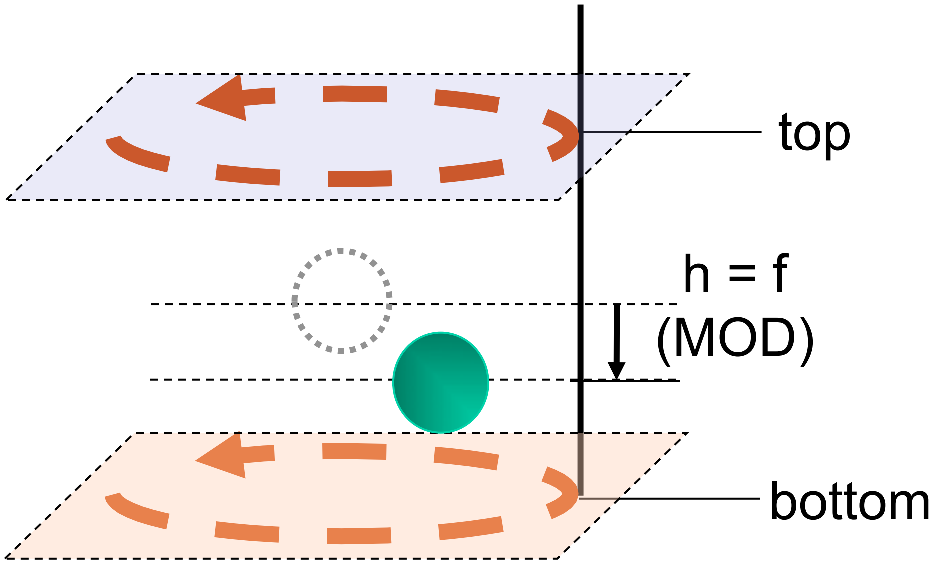


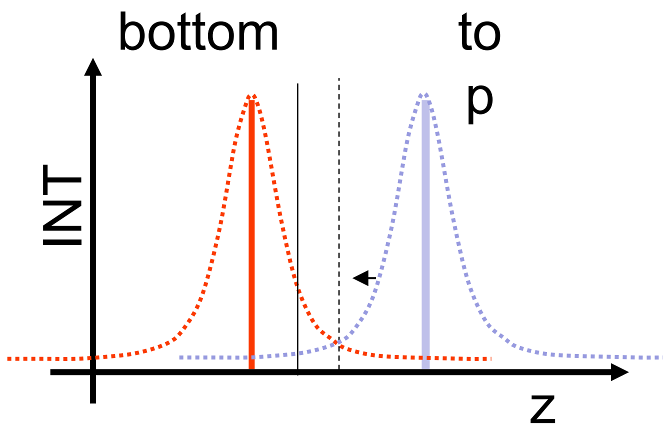
$$F(t) = \frac{2F_0/\pi}{1 + \frac{\lambda^2(z_p - z_s(t))^2}{w_0^4\pi^2}} \times \exp\left[-\frac{2[(x_p - x_s(t))^2 + (y_p - y_s(t))^2]}{w_0^2 + \frac{\lambda^2(z_p - z_s(t))^2}{w_0^2\pi^2}}\right] + B,$$

Estimate Position from Orbit

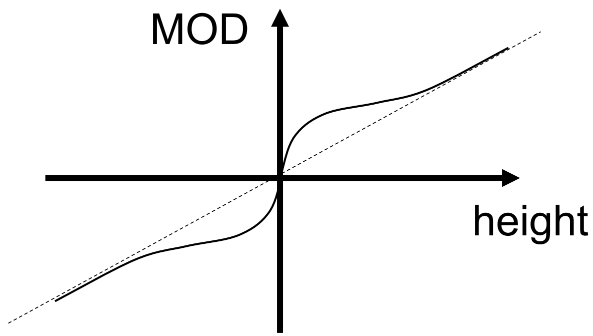
To locate the particle we need to know:

Angle, distance and **height** from center





$$MOD = 2\,(I_{top} - I_{bottom}) / (I_{top} + I_{bottom})$$



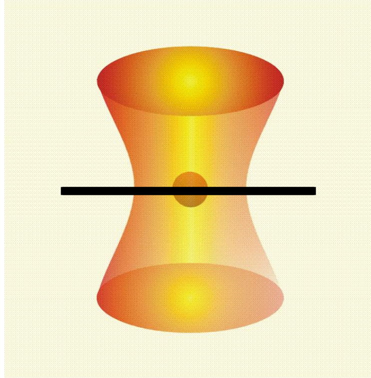
3-D Orbital Particle Tracking

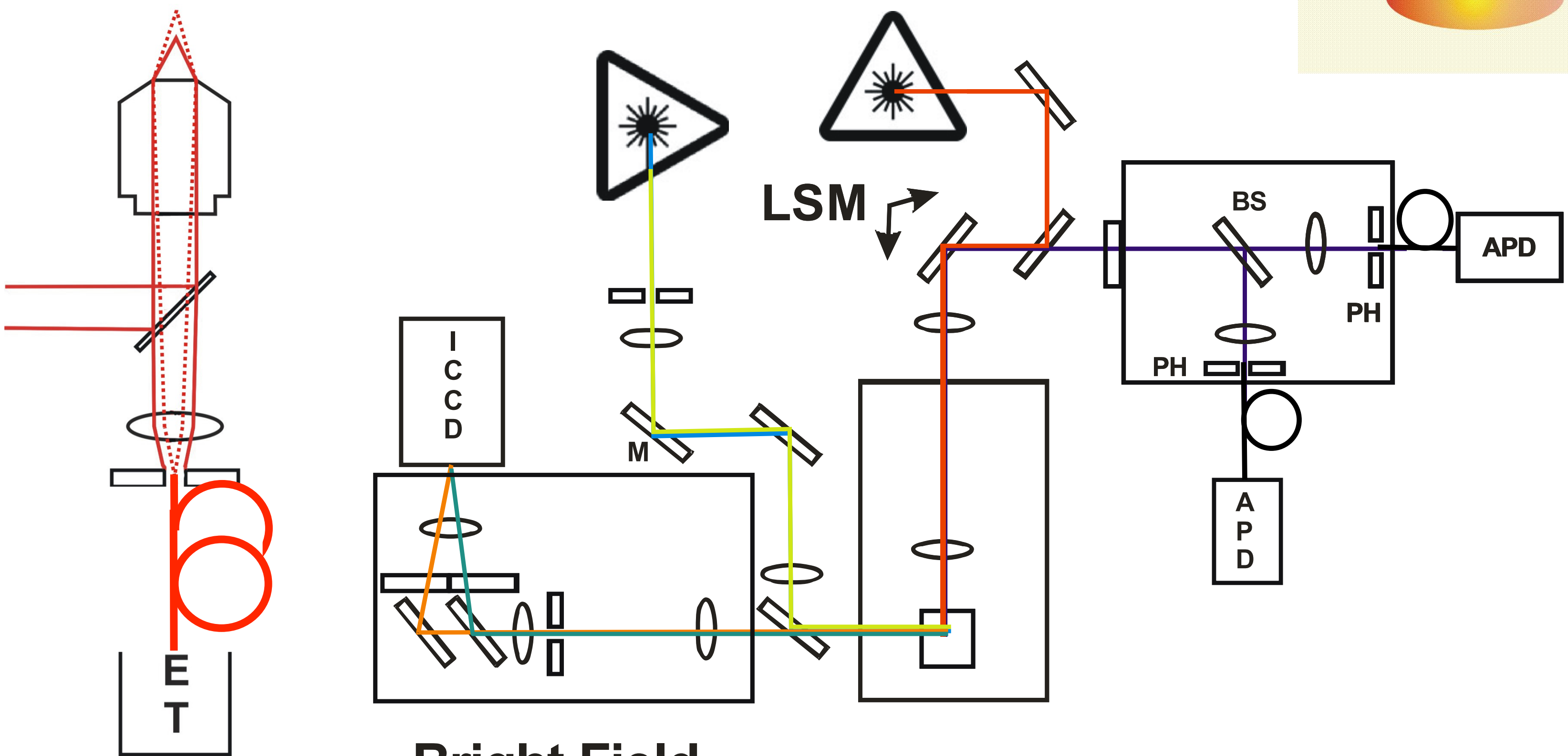
3D orbital tracking microscope (x,y & z)

with simultaneous bright field imaging

Focus, Det 1

Focus, Det 2





Bright Field

Tracking Algorithm

Initial position for the scanner $(x_s,y_s,z_s)_{t=0}$

Intensity profile

FFT

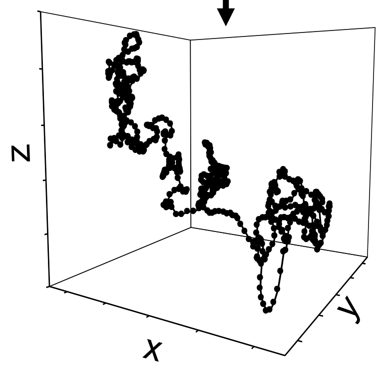
Intensity < threshold

Increase orbit radius

Particle position (x_p,y_p,z_p)

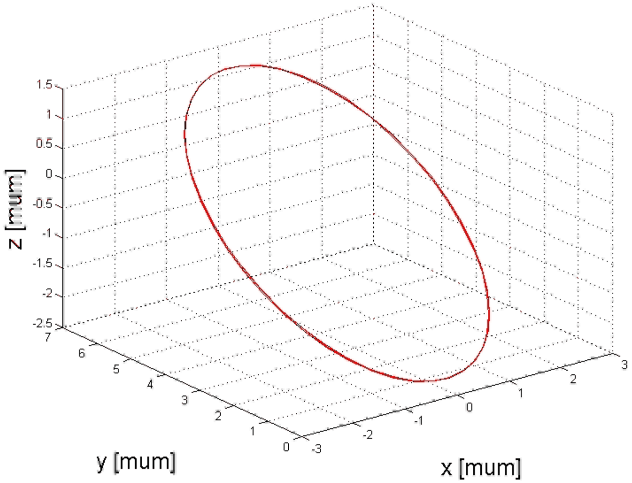
New scanner position

$(x_s,y_s,z_s)_t = (x_p,y_p,z_p)_{t-1}$

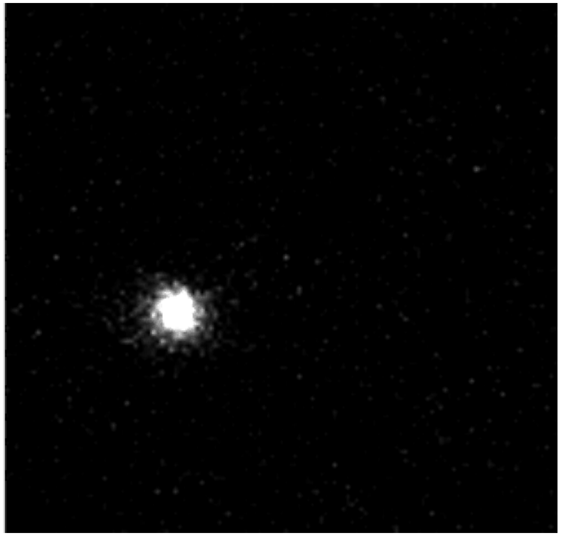


Simultaneous Wide-field Imaging

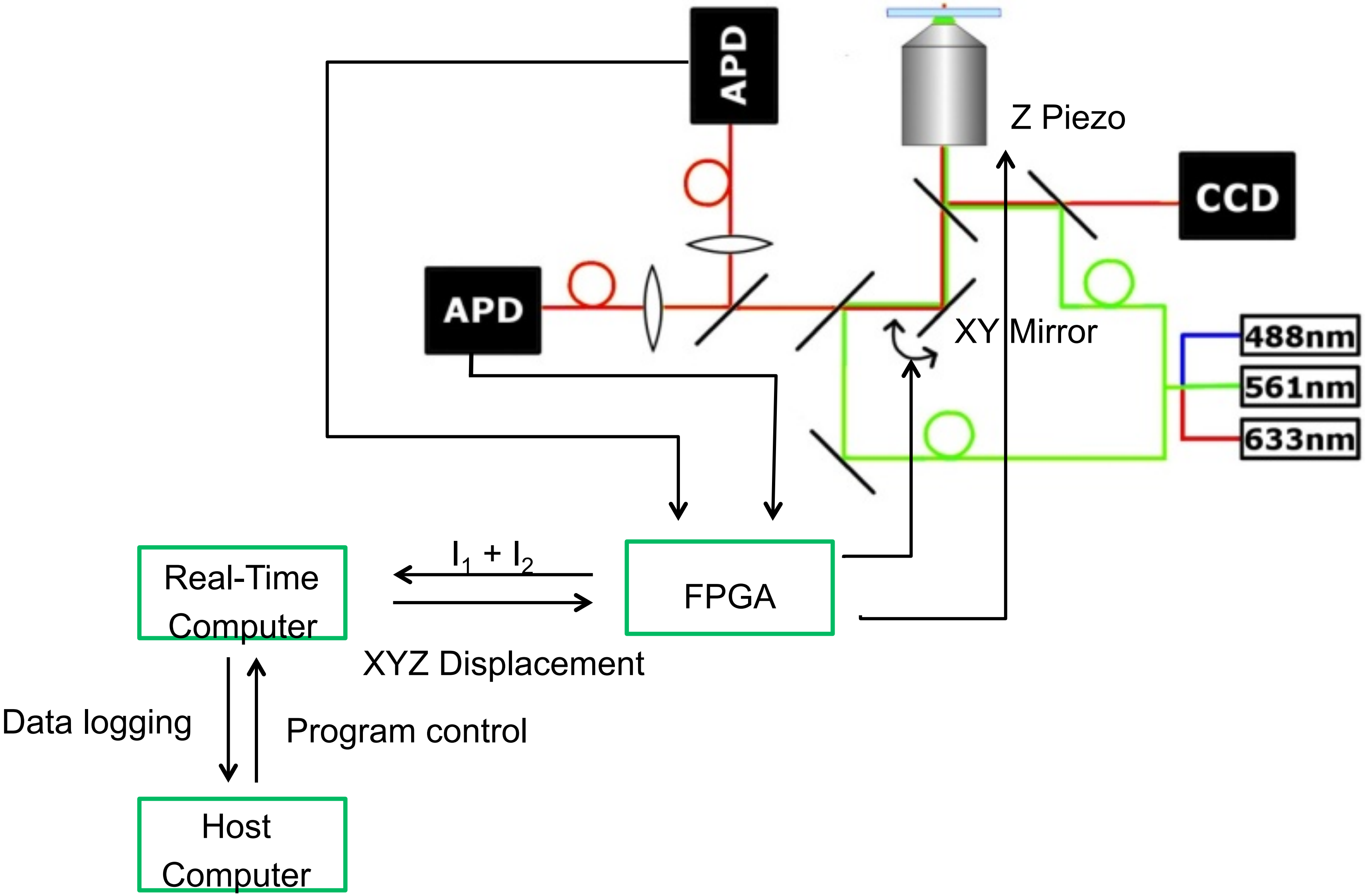
Wide-field without Tracking



Wide-field with tracking



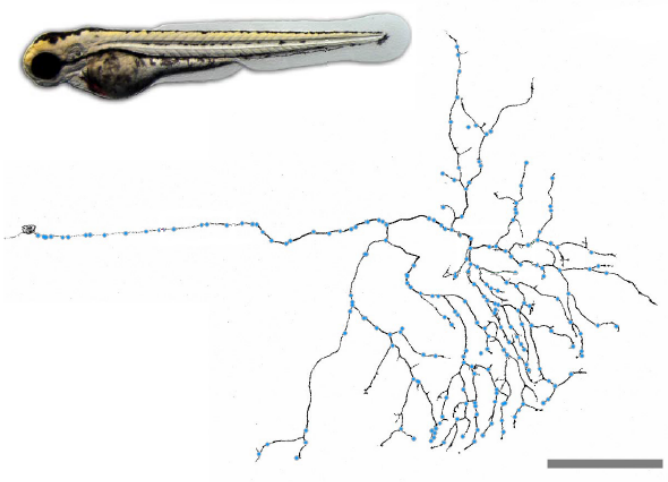
FPGA Control of Setup

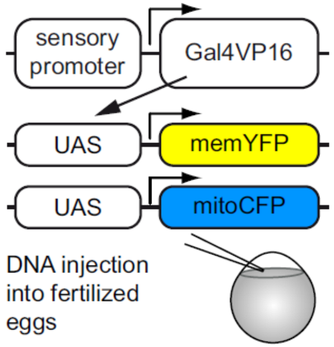


Charateristics of the 3D Orbital Tracking System

- Accuracy:
  - Lateral: ~ 5 nm
  - Axial: ~ 15 nm
- Acquisition rate: max. 500 Hz
- Tracking range: 200 µm x 200 µm x 100 µm
- Parallel Tracking: 4 particles
- Synchronization with acousto optical tunable filter
- Synchronization with widefield camera
- Long-range tracking over cms by repositioning the sample stage

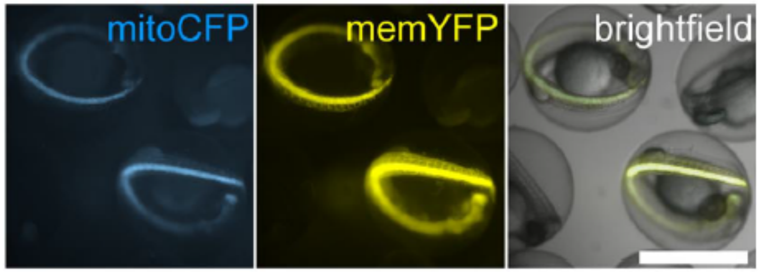
Tracking of Mitochondria in Zebrafish

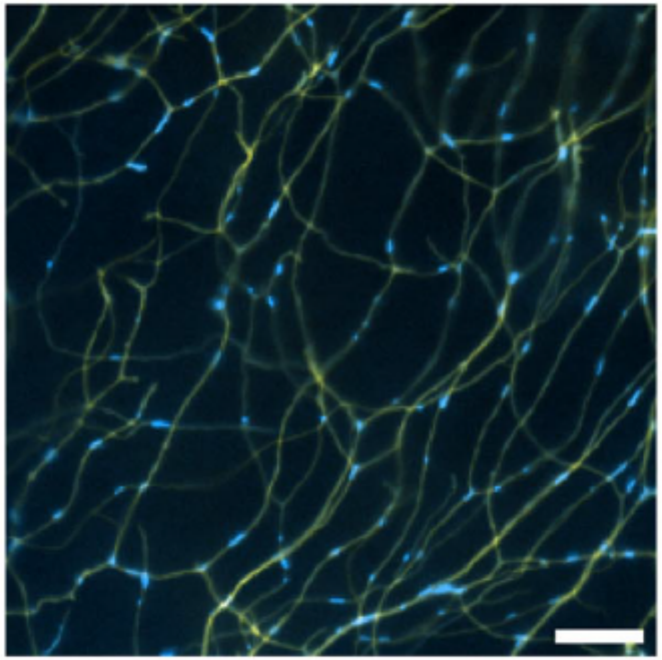




Fabian Wehnekamp

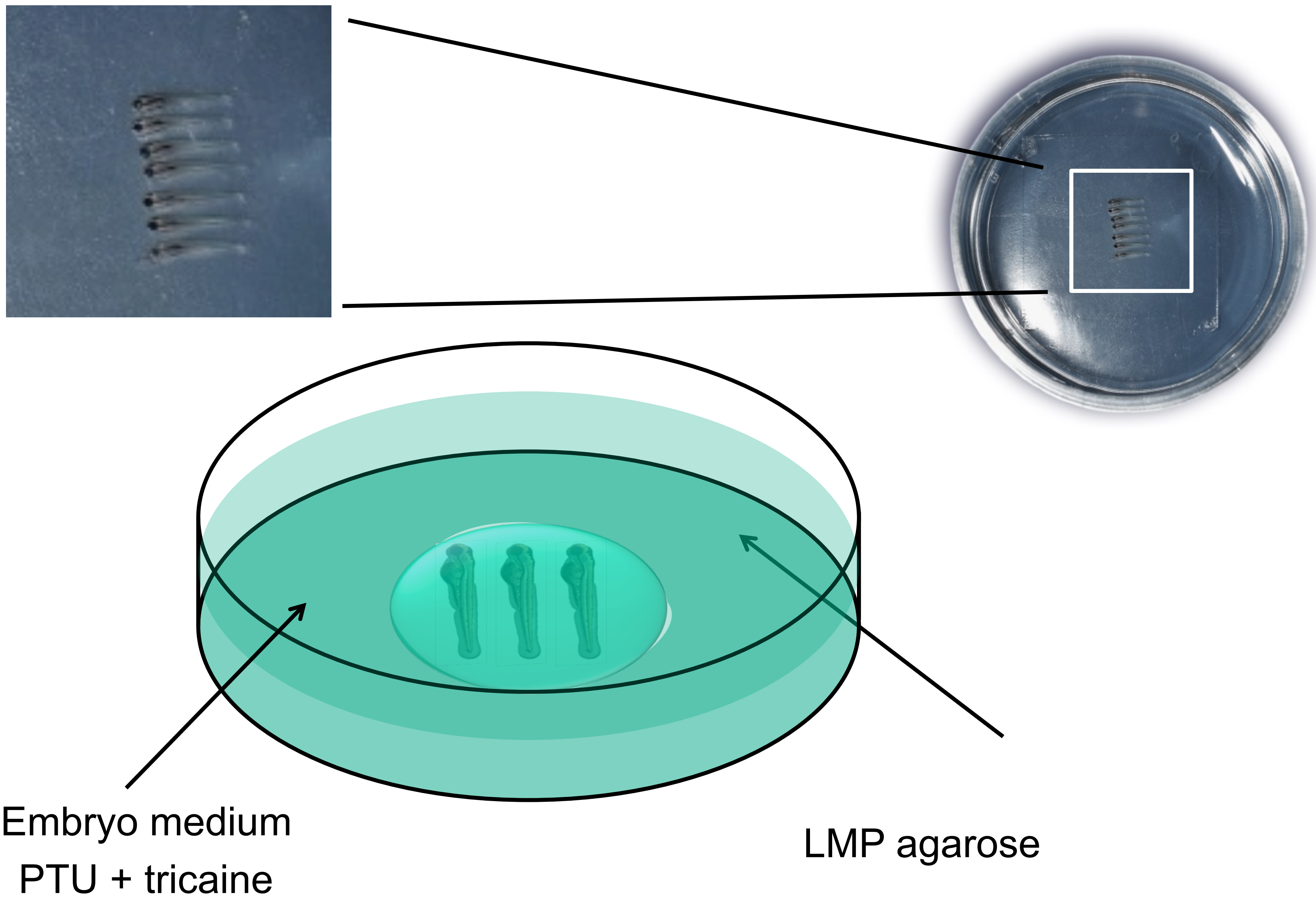
Thomas Misgeld
Gabriela Plucinska,
TU Munich





Plúcinska et al., *J Neurosci*, 2012

Tracking of Mitochondria in Zebrafish

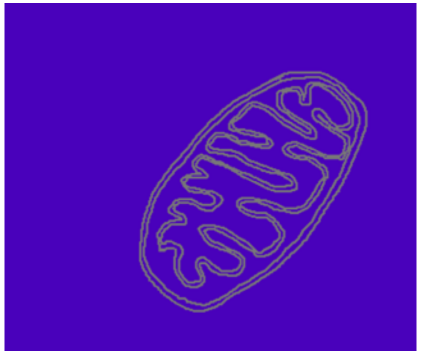


Tracking of Mitochondria in Zebrafish

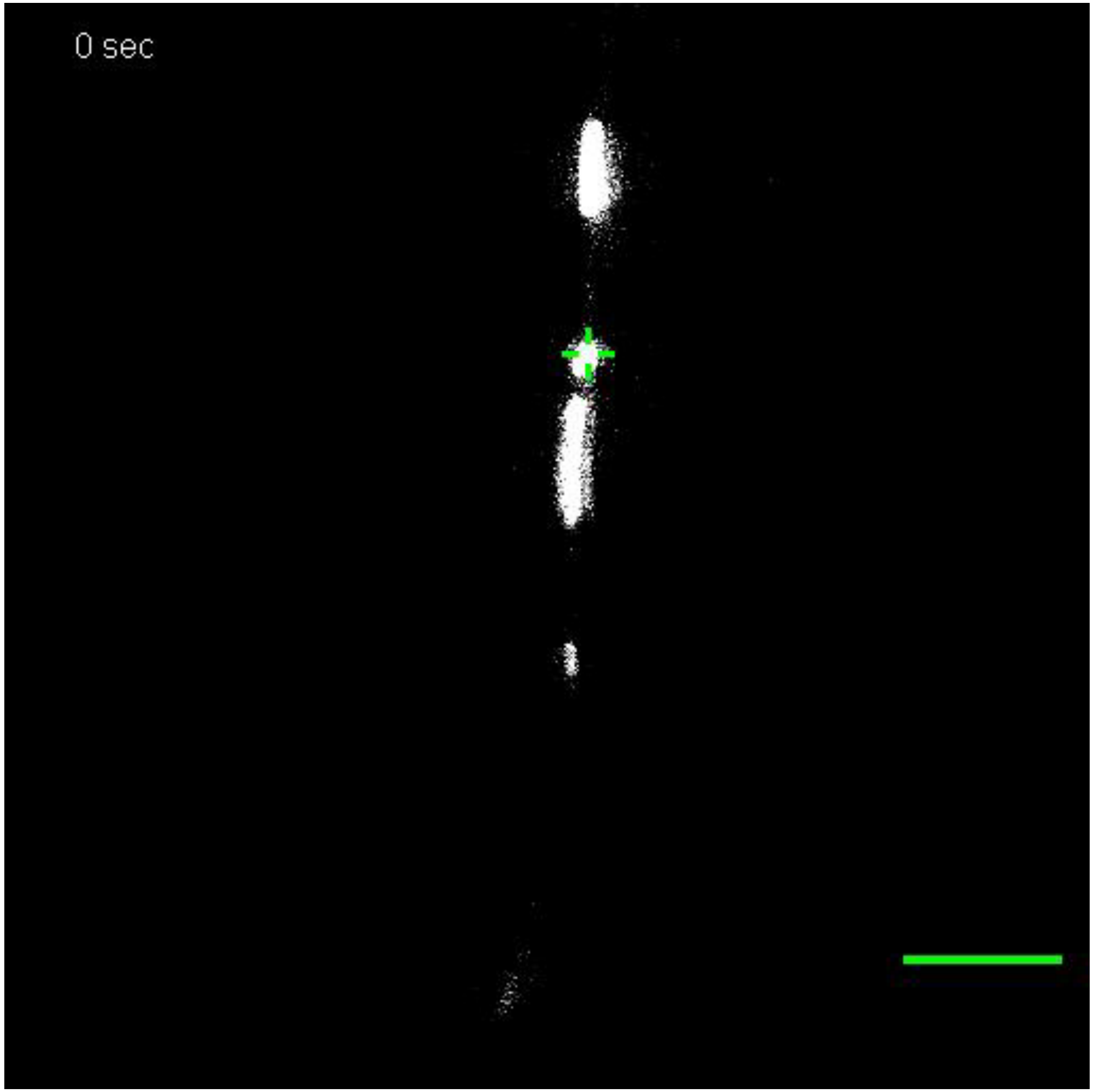
Widefield:

mitoTagRFP

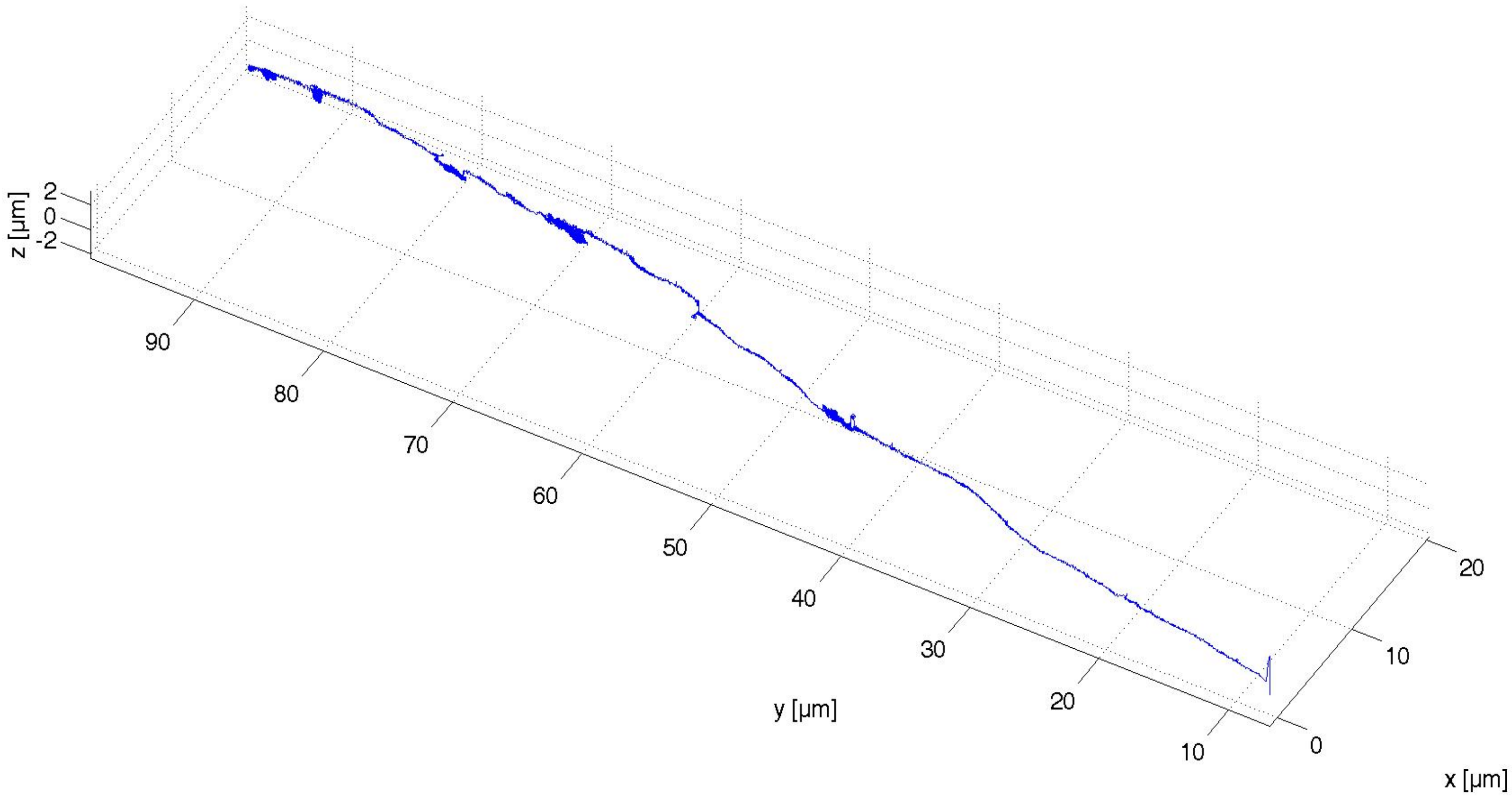
Tracking:



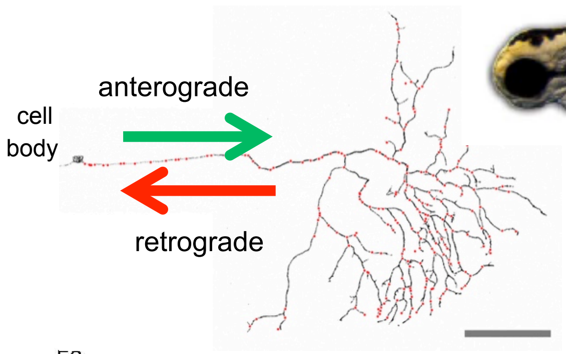
mitoPAGFP



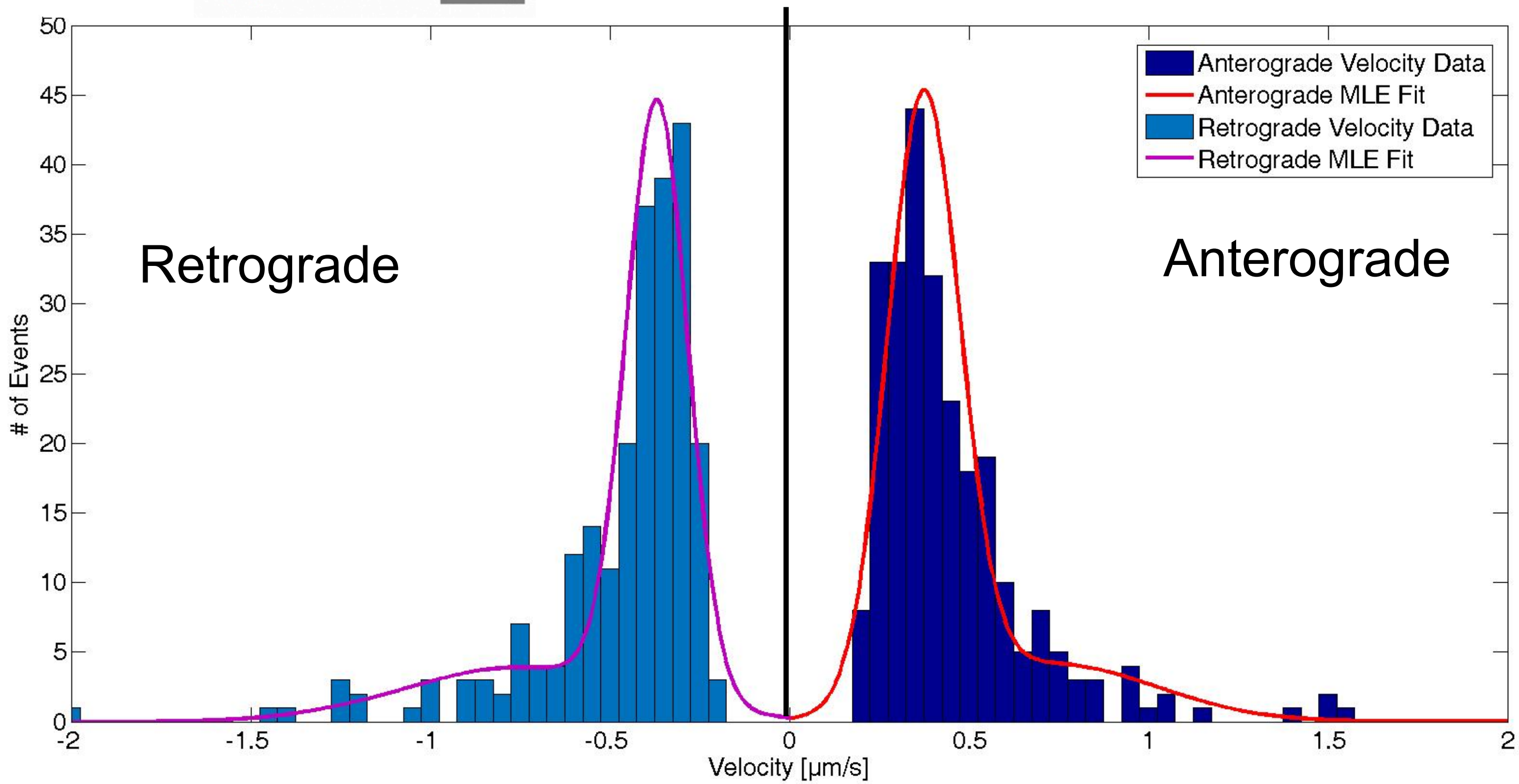
Tracking of Mitochondria in Zebrafish



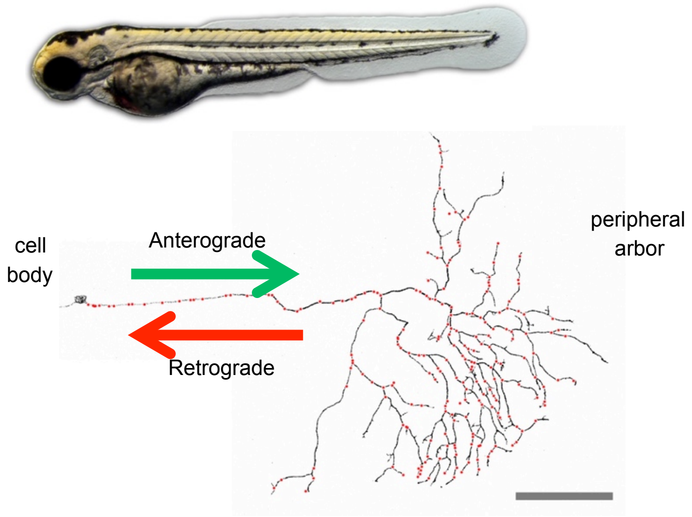
Analysis

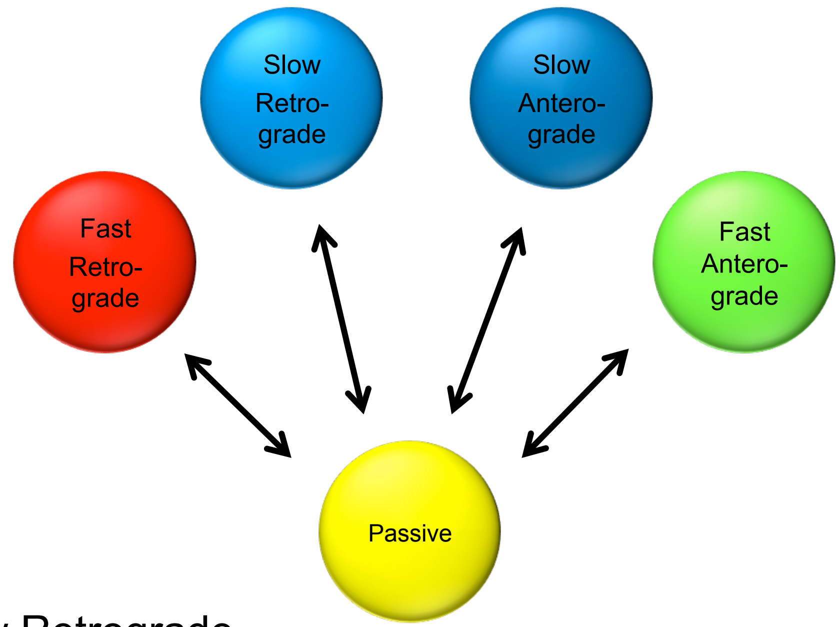


Fast Anterograde
Fast Retrograde
Slow Anterograde
Slow Retrograde



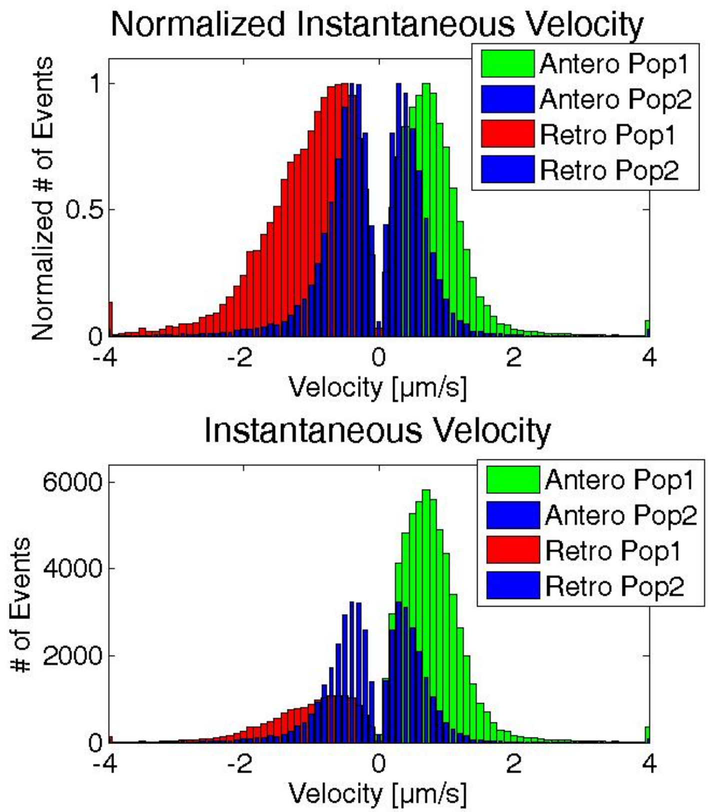
Tracking of Mitochondria in Zebrafish

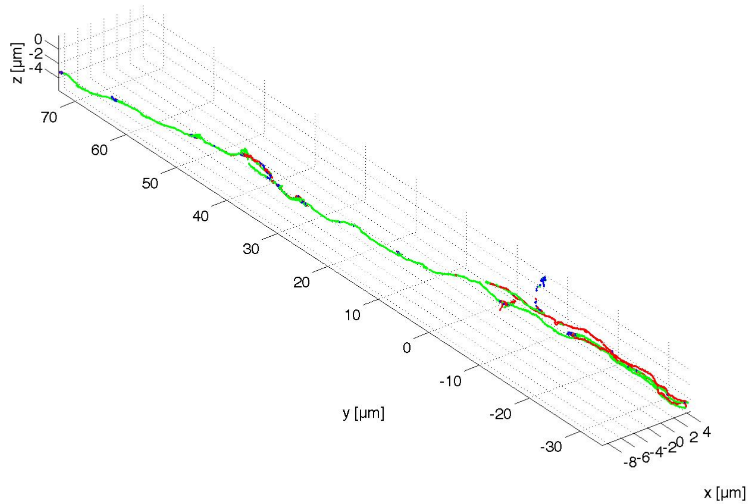




- Five different populations:
  - Fast Anterograde
  - Fast Retrograde
  - Slow Anterograde = Slow Retrograde
  - Passive
- No direct transitions between moving populations
- No polarization change during long movements in stem axon

Tracking of Mitochondria in Zebrafish





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