Dynamics of the Cell Interior – from Insight to Control

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„Physics of Life Processes"- Area

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Leiden, The Netherlands

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„3D NanoCell"

Fraunhofer Institute
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Neunerplatz 2
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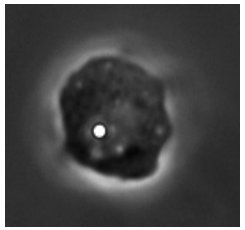
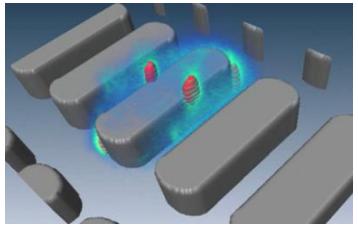
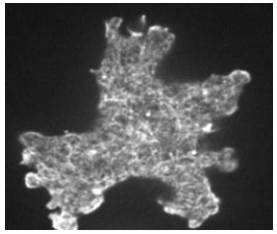
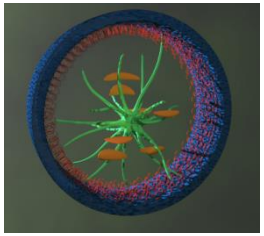
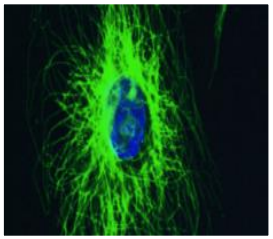
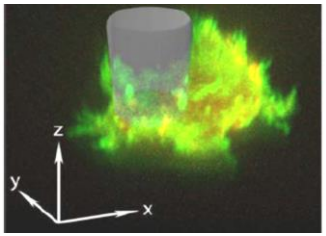







Current Positions:

Professor of Biophysics

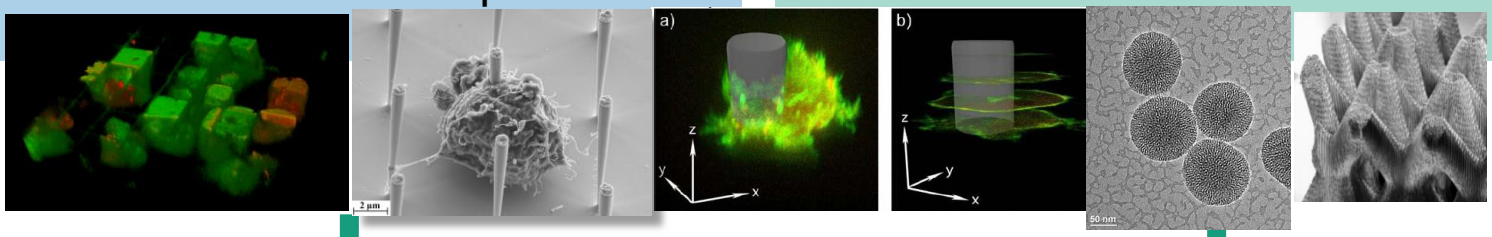
Basic Research

Control of Cell Functions
Cells on Nanostructures and -surfaces
Advanced Cell Microfuidics
Cells in Magnetic Tweezers
Intracellular Transport

Head of Fraunhofer-Attract `3D NanoCell´

Applied Research

3D-Nanostructured Cell Assays
3D-Scaffolds
Multifunctional Nanoparticles
Smart Implants
Tissue Engineering



<u>Joint Research Group:</u>
Basic Research Innovations are shuttled directly to Industry: Publications and Patents

Motivation

Investigation and control of live cell interactions with nanostructured surfaces, coatings and chemical agents (drugs) for novel diagnostic methods, 3D-scaffolds for tissue engineering and smart implants

(1) Diagnostics-Assay for Pharmaceuticals

Nanostructured 3D-scaffolds with authentic cell systems as standard diagnostics assay for drug screening

(2) Structure-optimized Implantsurfaces

Combination of standard and novel materials

(3) 3D-Scaffolds for Tissue Engineering

Bottom-up nanostructured, porous Scaffolds for in-vivo- und in-vitro-regeneration of tissue and organs

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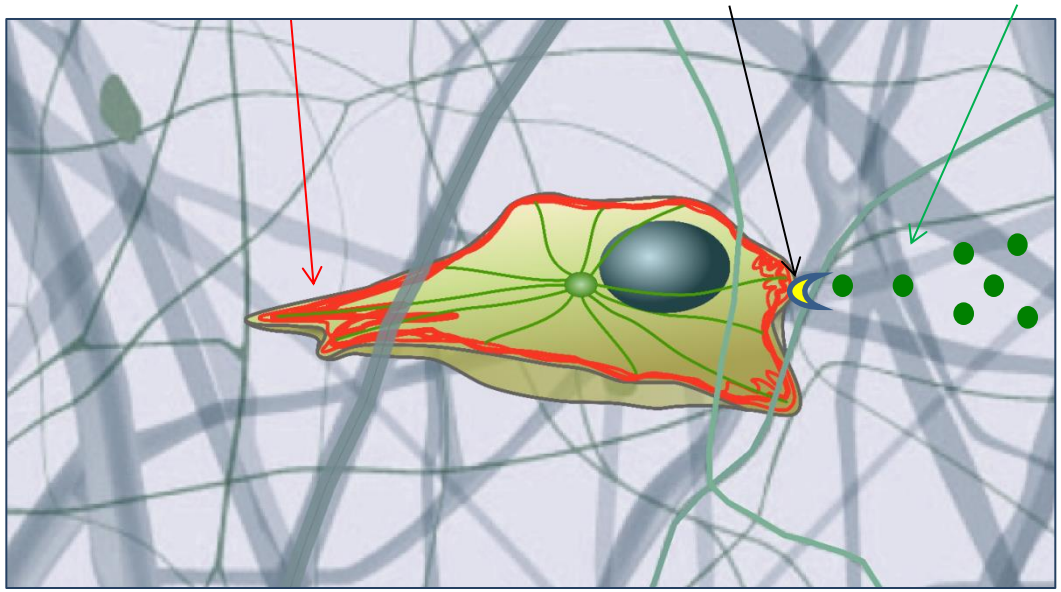
Cells in 3D environments

3D

Actin (nm-µm) Cell Receptors (~nm) Gradient (nm-µm)

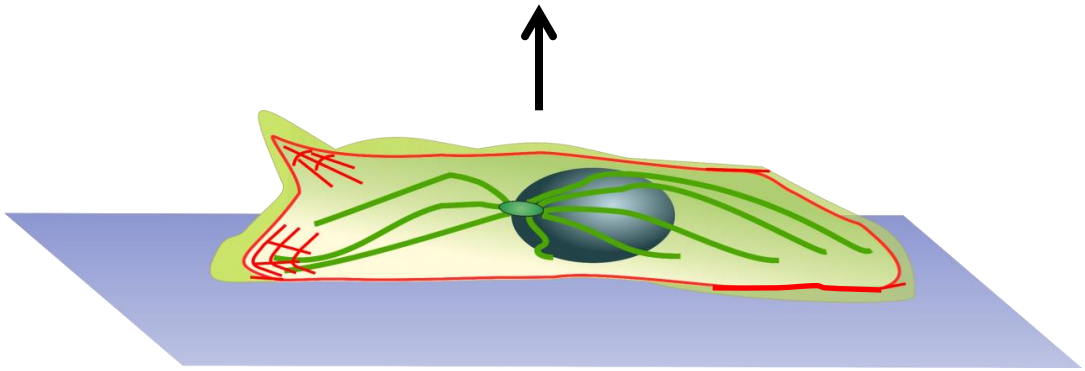


Dynamic processes on time scales from ms to hours

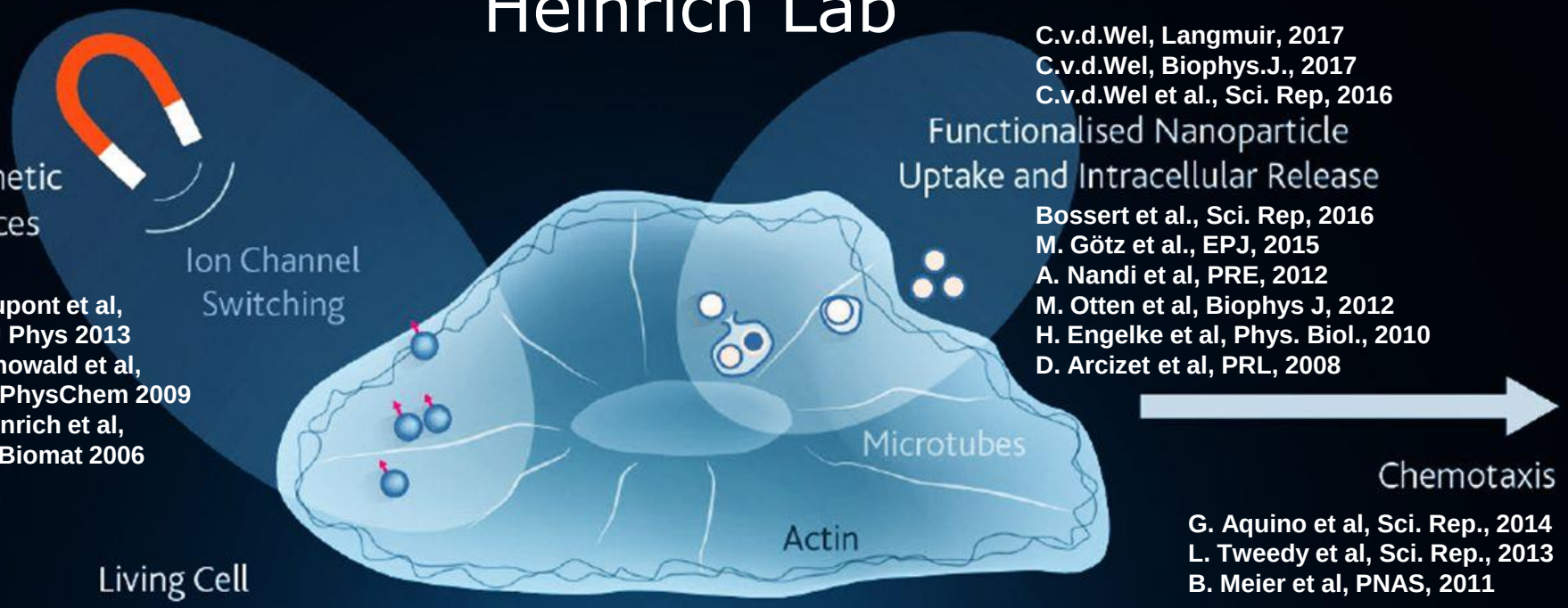
Important for

- Tissue Engineering
- 3D Drug Screening
- Organ/ Lab on a chip

2D



Heinrich Lab



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D. Arcizet et al, PRL, 2008

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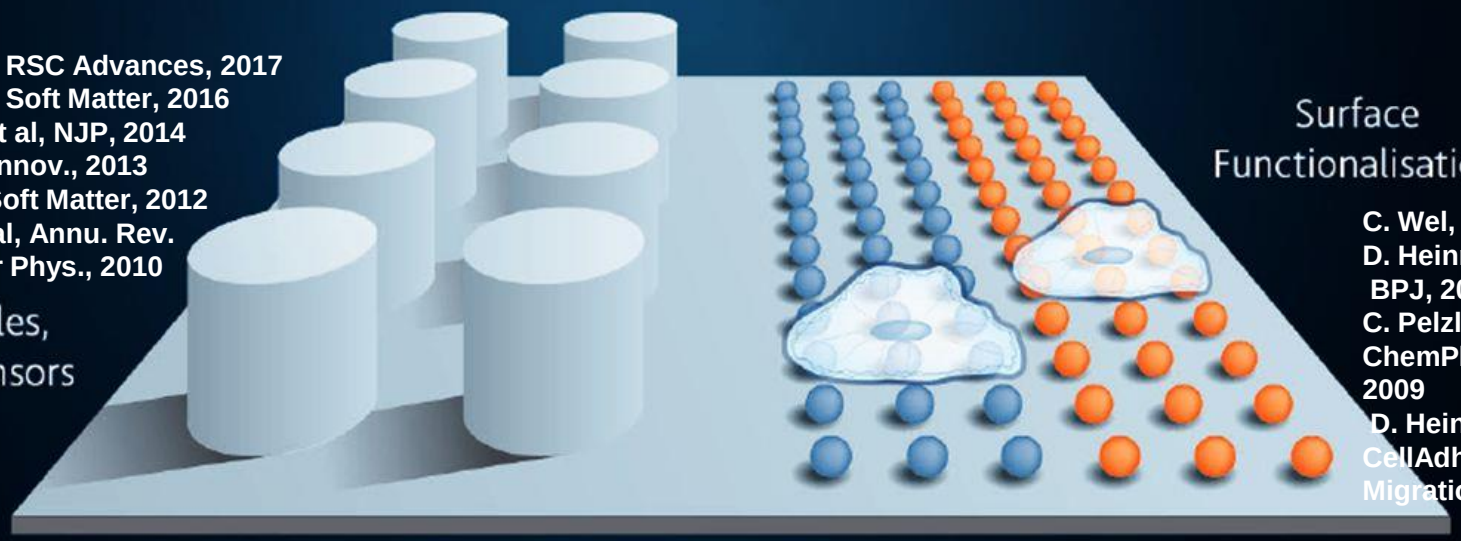

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D. Heinrich, Int. Innov., 2013
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E. Sackmann et al, Annu. Rev. Condens. Matter Phys., 2010

C. Wel, Sci. Rep, 2016
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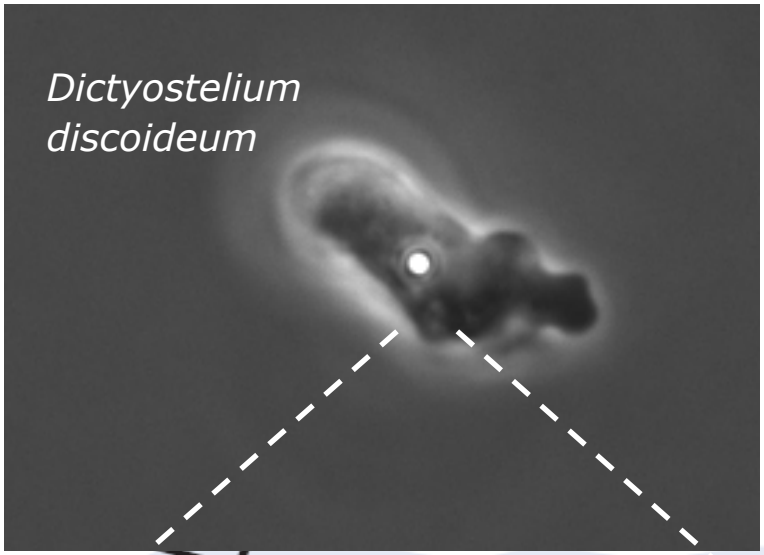
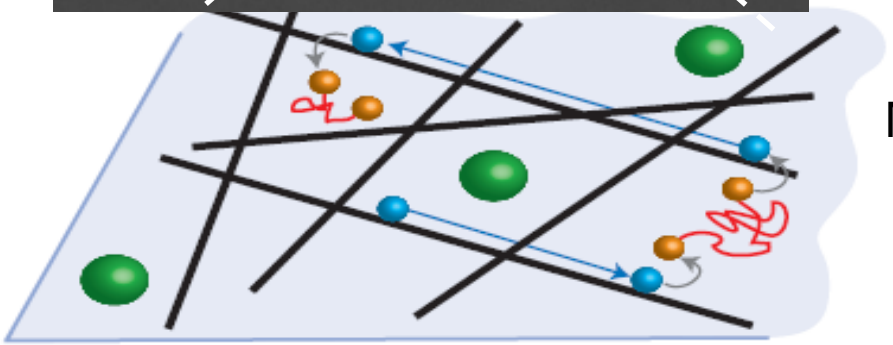
D. Heinrich et al, CellAdhesion& Migration, 2008

The Cellular Cytoskeleton as an Active Crowded Network

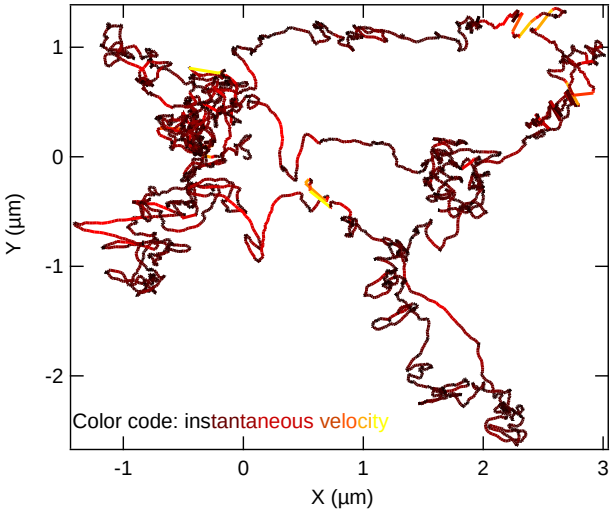
Are there Systematic Features in this Complex Behavior?



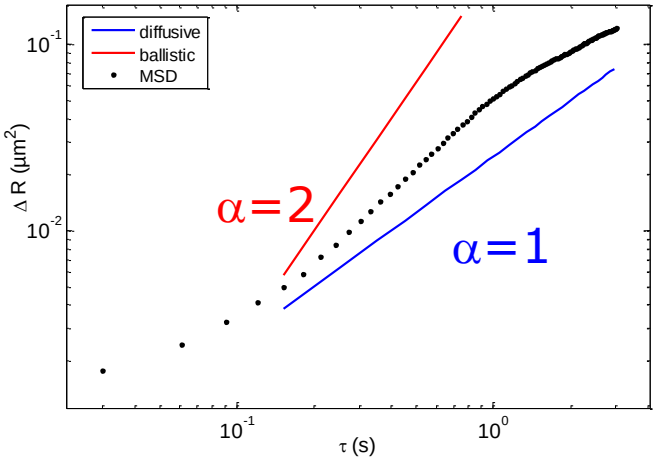


Loverdo et al. Nature Physics 2008

Particle Tracking →



Most simple analysis: MSD →

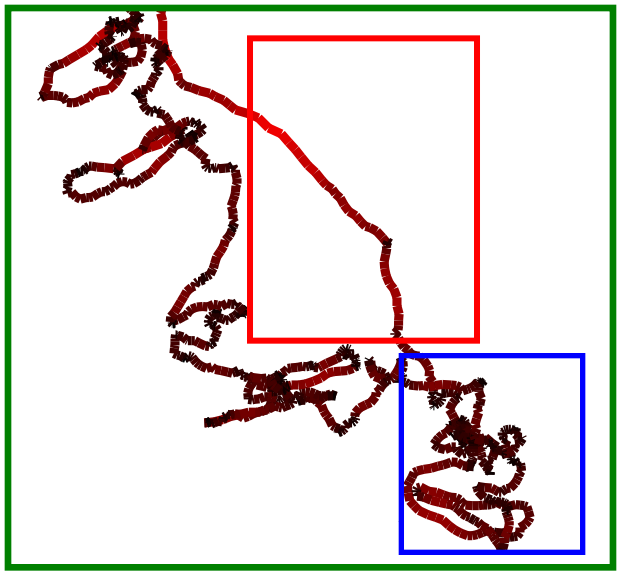


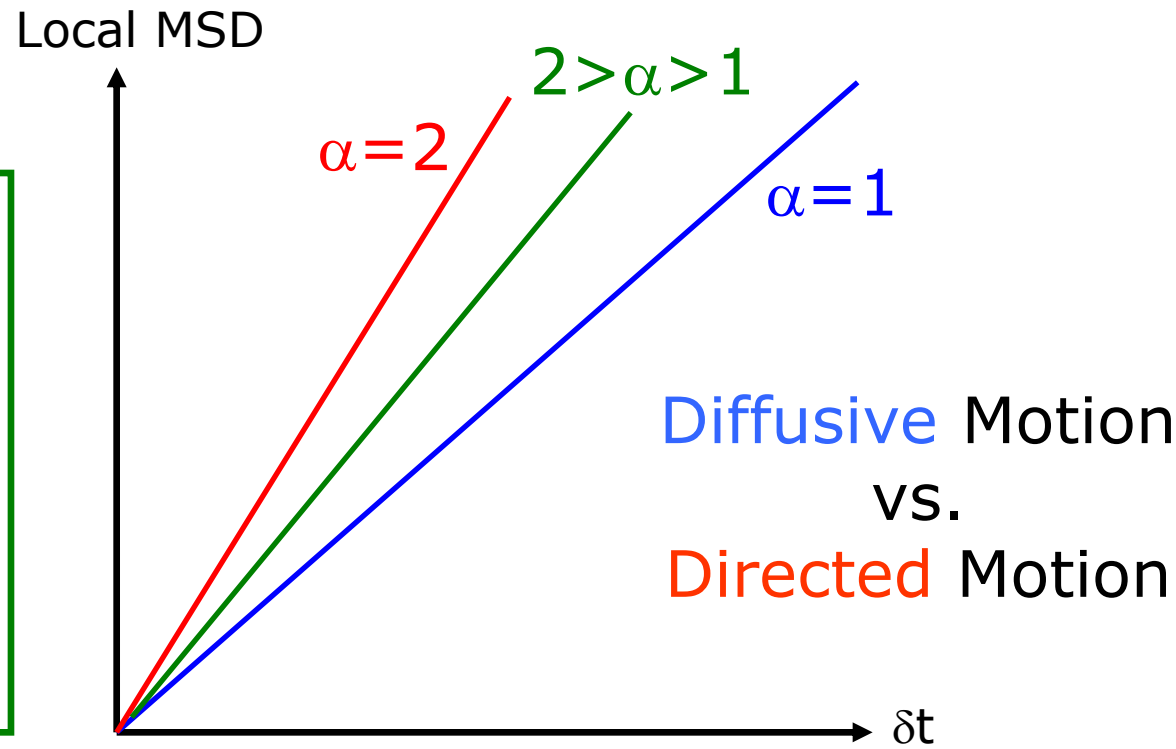
Local Analysis of Intracellular Tracer Motion

Short Time Window slides over Long-term Tracer Track:

-> determine mean-squared displacement (MSD) and correlation function of velocity

Intracellular
Particle Trace





Local Mean Squared Displacement Algorithm

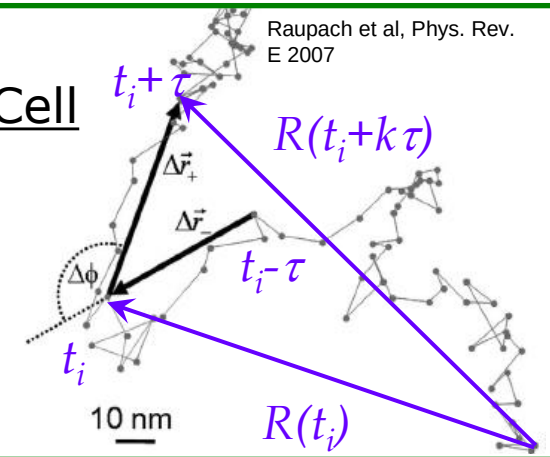
Condition 1:

Local Mean Square Displacement of Bead Motion in Cell

$$\Delta R_t^2(\delta t) = \langle (\mathbf{R}(t'+\delta t) - \mathbf{R}(t'))^2 \rangle_{-\frac{T}{2}<t'<\frac{T}{2}} = A\,\delta t^{\alpha}$$

if $\alpha=1$, particle undergoes Brownian Diffusion

if $\alpha=2$, particle is actively transported



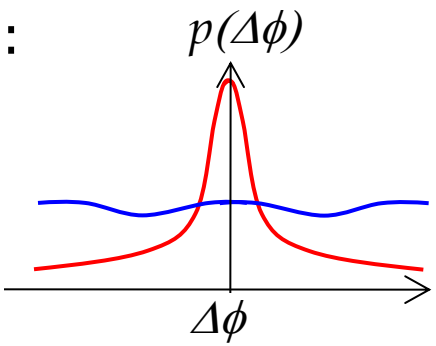
Condition 2:

Angle Correlation Function for Directional Persistence:

$$\Delta\phi_t(\delta t) = \langle (\phi(t'+\delta t) - \phi(t'))^2 \rangle^{1/2}_{-\frac{T}{2}<t'<\frac{T}{2}}$$

if purely Brownian Motion: $\Delta\varphi(\tau) = \text{random}$

for directional Movement: $\Delta\varphi(\tau) \approx 0$



Probability for Active or Passive Intracellular Transport

$$p_A = \begin{cases} 1 & \text{if } \alpha = 2 \pm \sigma_\alpha \wedge \Delta\phi = 0 \pm \sigma_\phi \\ 0 & \text{otherwise} \end{cases}$$

→ Active Transport State

Passive Diffusion State

M. Götz,… EPJ, 2015; A. Dupont,… NJP, 2013; A. Nandi,… PRE, 2012; M. Otten,… Biophys J, 2012; D. Arcizet,… PRL, 2008

Temporal analysis of motion phases in living cells

Motion of endocytosed bead in Dictyostelium discoideum can be described by a two-state model

Algorithm-dissected track

Angle variation (std)

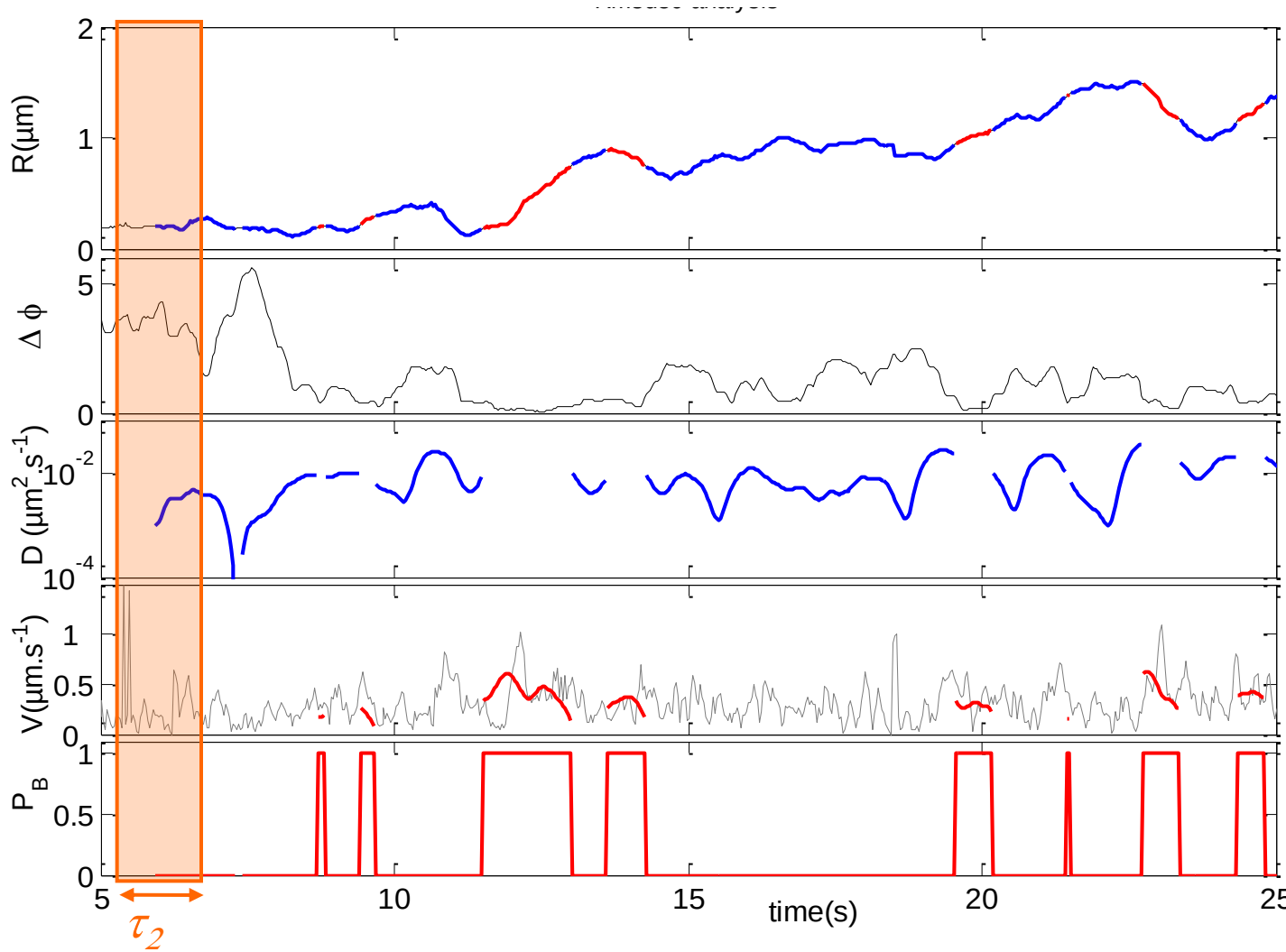
Diffusion coefficient

$$D_i = A_i / 4$$

Velocity

$$v_i = \sqrt{A_i}$$

Probability ballistic phase



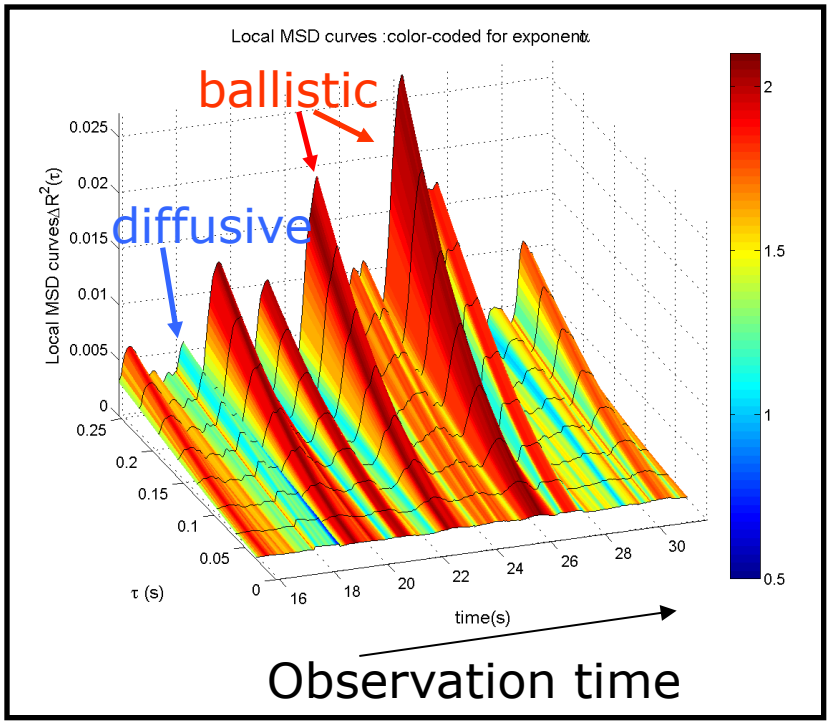
M. Götz,… EPJ, 2015; A. Dupont,… NJP, 2013; A. Nandi,… PRE, 2012; M. Otten,… Biophys J, 2012; D. Arcizet,… PRL, 2008

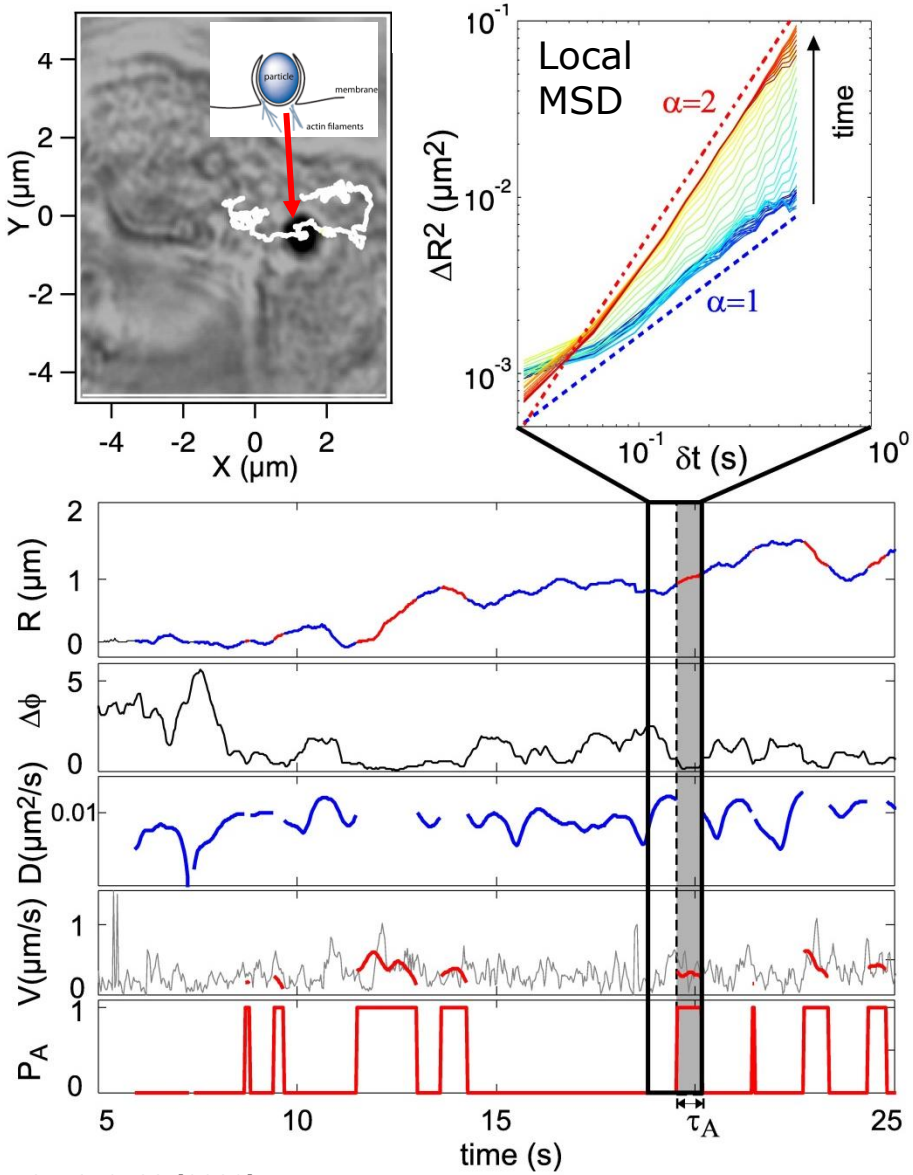
Local MSD Analysis of a Tracer Bead in a Living Cell

Local MSD curves retrieved from a bead in a living cell exhibit:

- clear power-law behavior
- accumulation at $\alpha=1$ and $\alpha=2$

Active States ↔ Passive States





D. Arcizet, B. Meier, E. Sackmann, J. O. Rädler, and D. Heinrich, Phys. Rev. Lett. 101, 248103 (2008)
A. Dupont, M. Gorelashvili, V. Schüller, F. Wehnekamp, D. Arcizet, Y. Katayama, D.C. Lamb and D. Heinrich, New Journal of Physics 15, 075008, 2013

Analysis of Active and Passive Noise Spectra in Living Cells

Active Motion States:

narrow v-distribution with v_A=0.39 µm/s

Peaks in v-distribution

$v_n = n \cdot v_1$ (v_1=0.225 µm/s)

-> discrete behavior of active v-distribution

Diffusive States:

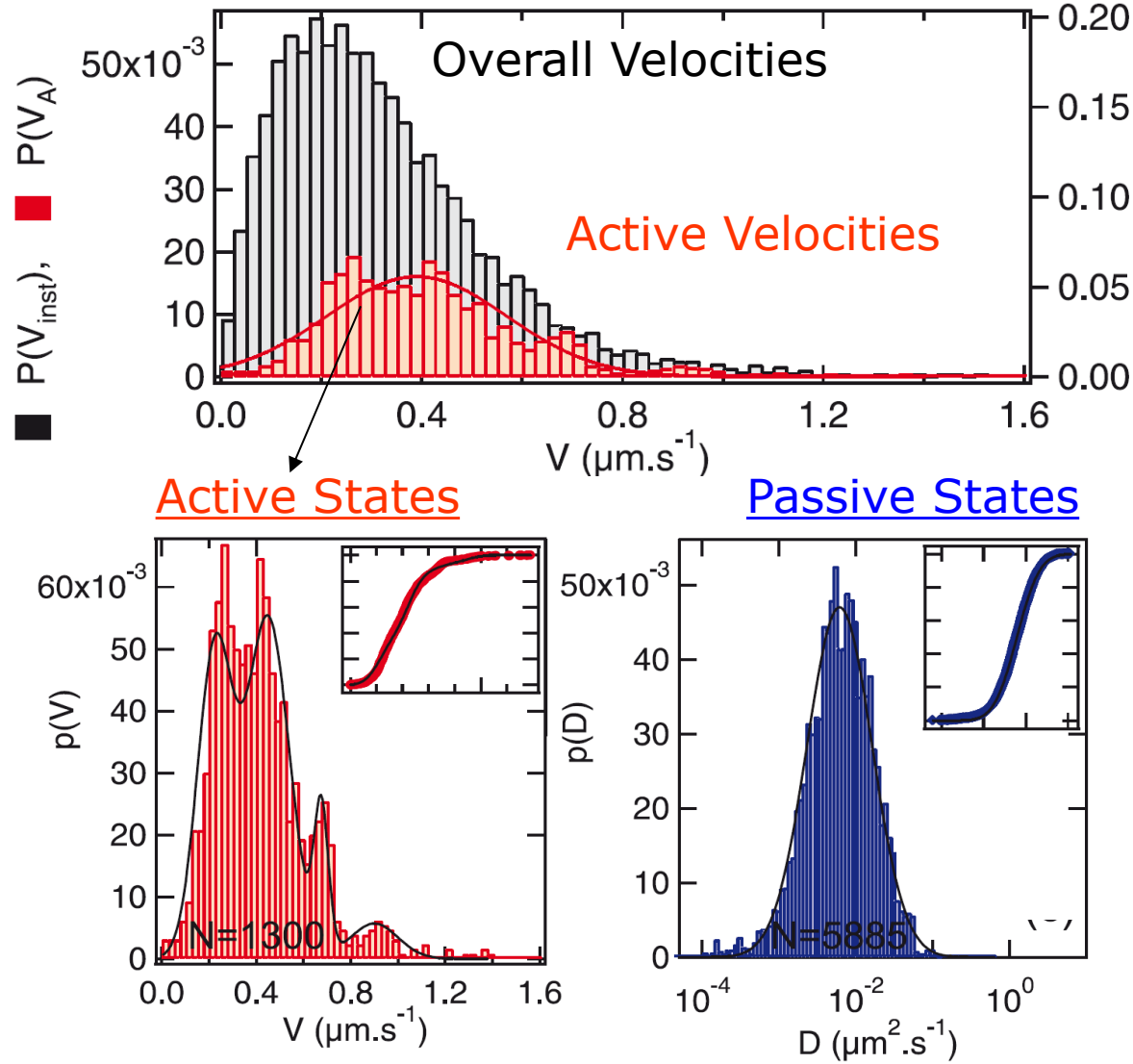
log-normal D-distribution with $D_P = 6.1 \cdot 10^{-3}$ µm²/s

Cytoplasm viscosity:

$\eta_c = k_B T / 6\pi R D_p = 5 \cdot 10^{-2}$ Pa.s

Molecular Motor Force:

$F = 6\pi\eta_c R v_1 = 0.15$ pN

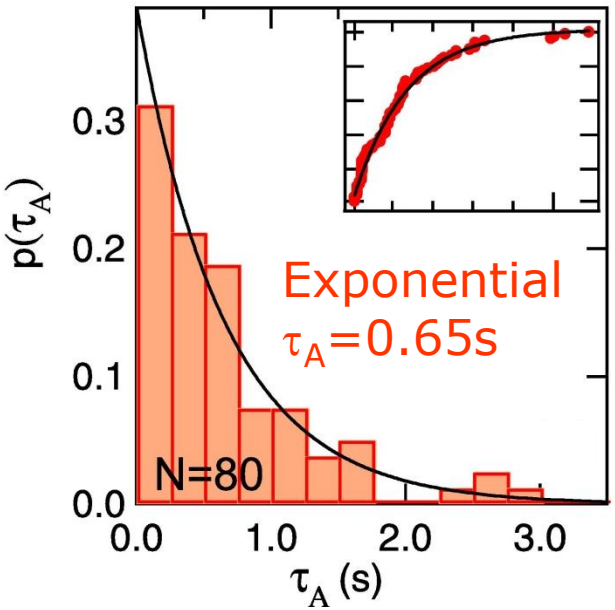


M. Götz et al., EPJ 2015 A. Dupont, New J Phys 2013 A. Nandi et al, PRE 2012 M. Otten et al, Biophys J 2012 D. Arcizet et al, PRL 2008

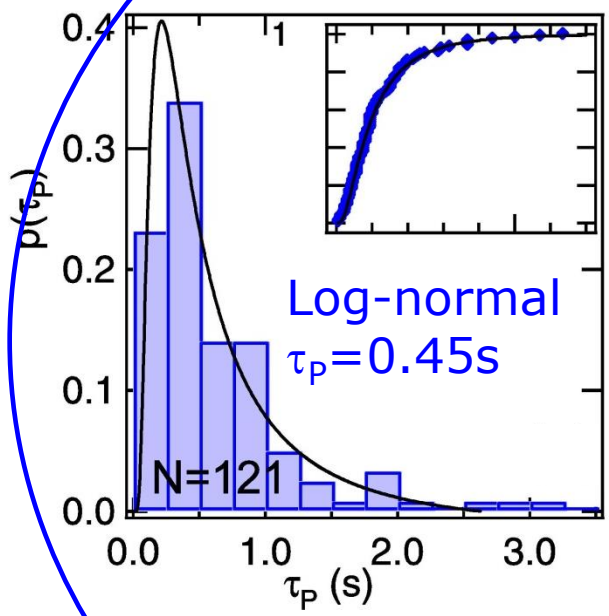
Analysis of Active and Passive Noise Spectra in Living Cells

A-state and P-state durations in comparison:

Active States



Passive States



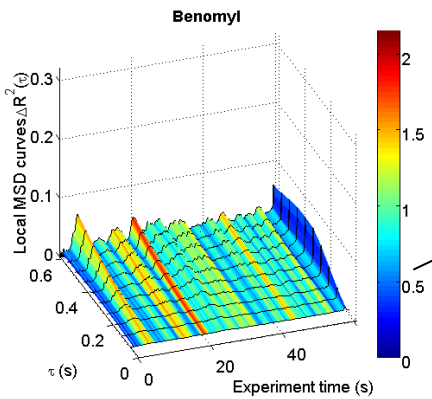
Superposition of processes of various origins and time scales:

- free bead diffusion
- mechanical bead blocking
- transient filament and organelle contact
- binding time of motors to filaments

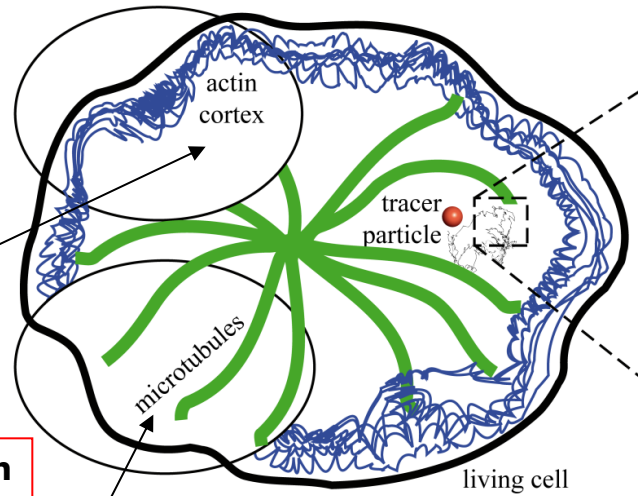
D. Arcizet, B. Meier, E. Sackmann, J. O. Rädler, and D. Heinrich,
Temporal analysis of active and passive transport in living cells, Phys. Rev. Lett. 101, 248103 (2008)

Influence of Cytoskeleton components on Intracellular Transport

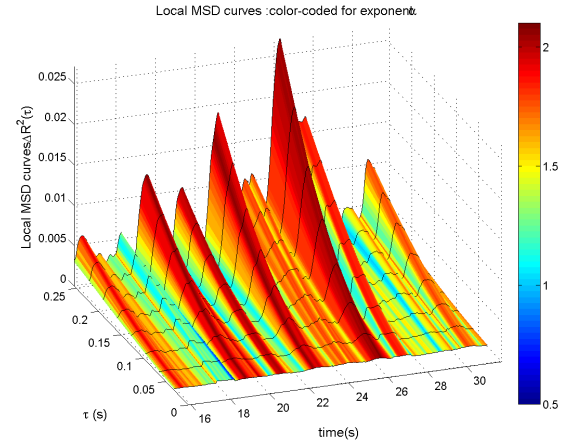
without microtubules



Treatment with Benomyl depolymerizes microtubules

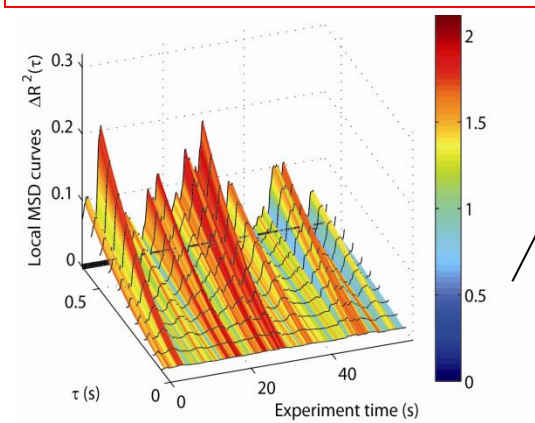


with microtubules



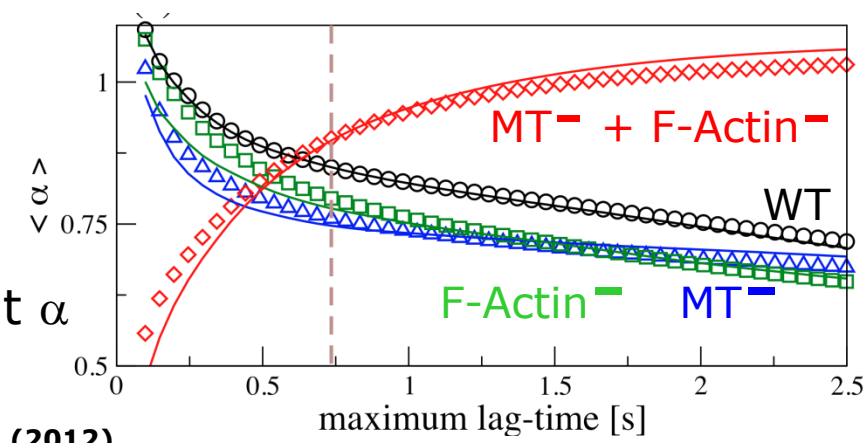
J. Mahowald, D. Arcizet, D. Heinrich
ChemPhysChem, 2009

with microtubules, no F-actin



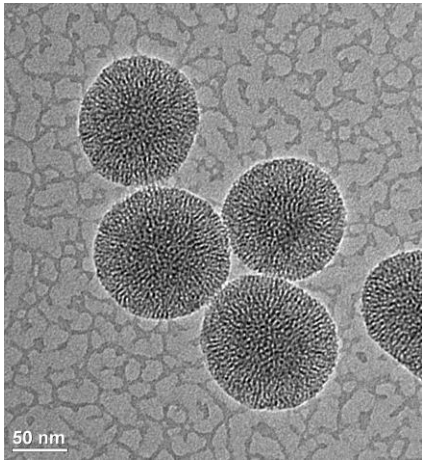
Treatment with Latrunculin A depolymerizes actin cortex

Lag time Dependent MSD Coefficient α

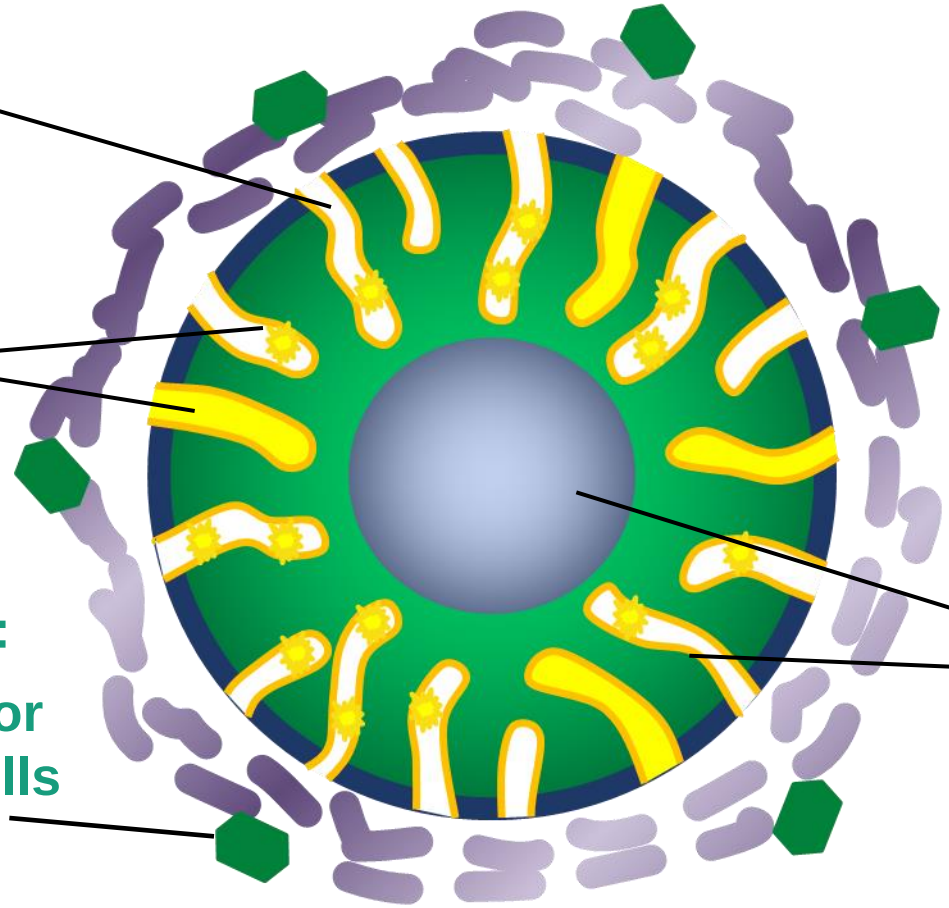


M. Otten, A. Nandi, B. Lindner and D. Heinrich, Biophys. J 102, 758 (2012)
A. Nandi, D. Heinrich and B. Lindner, PRE 86, 021926 (2012)
B. D. Arcizet, B. Meier, E. Sackmann, J. O. Rädler, and D. Heinrich, Phys. Rev. Lett. 101, 248103 (2008)

Design of the Nano-container Multishell - Concept



Mesoporous Particles



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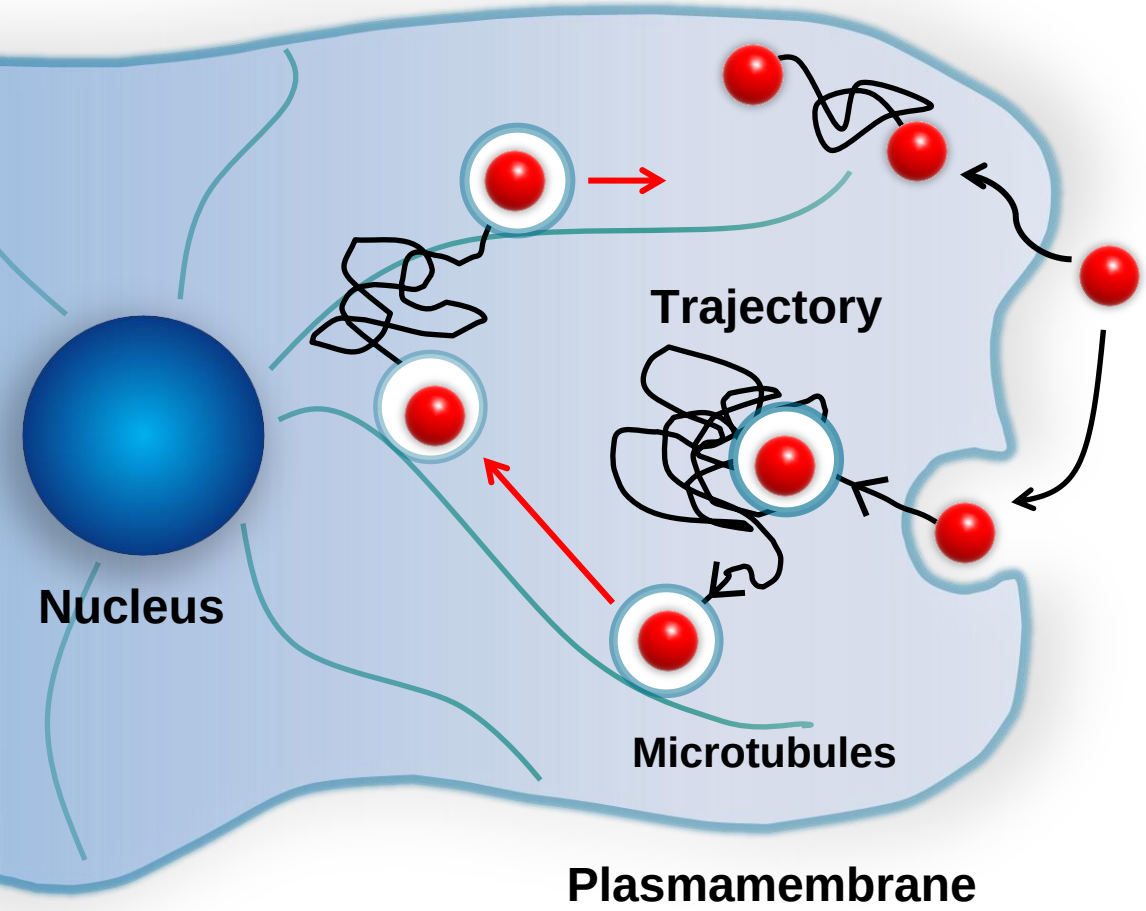
Single particle tracking in cells



Following uptake in to the cytosol:

- **<u>diffusive</u> transport**

Following uptake in vesicles:

- **<u>diffusive</u> transport**
- **<u>active</u> transport**

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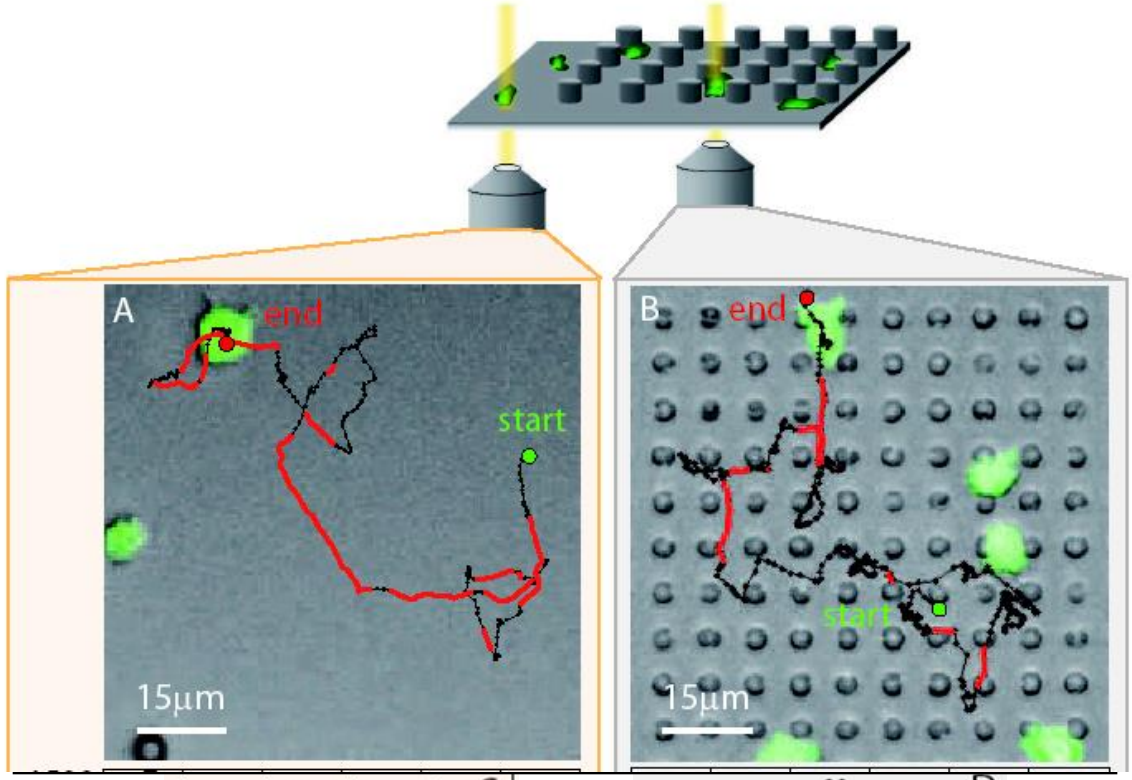
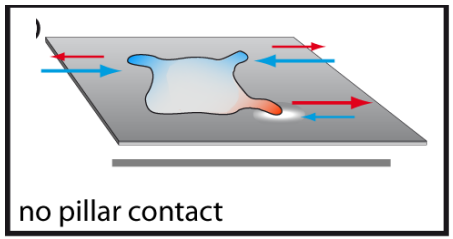
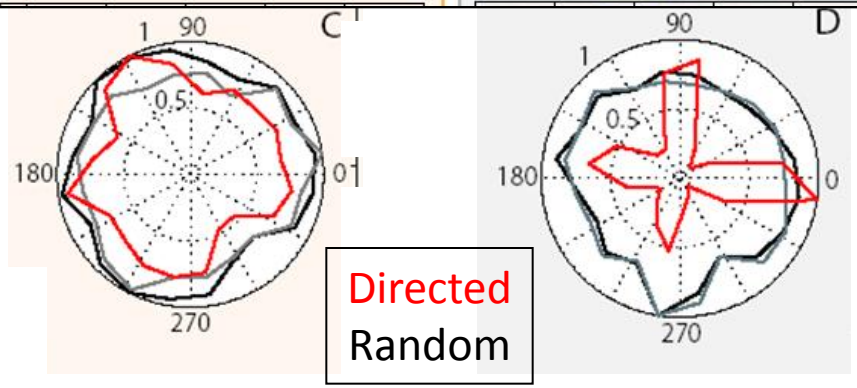
Quasi-3D Structures Control Cell Migration

Cell Motion Analysis by Local MSD (PRL 2008)

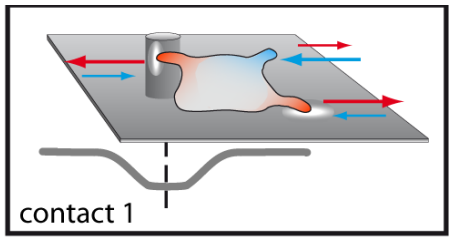


Control of Migration Direction and Velocity in Quasi-3D





Directed
Random



Soft Matter, 2016 + Nature Communications, subm.

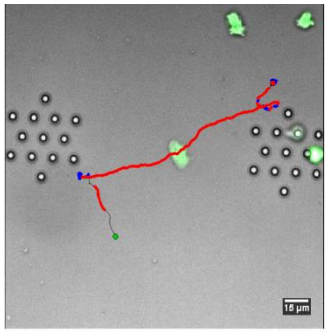
Soft Matter 8(5):1473 (2012)

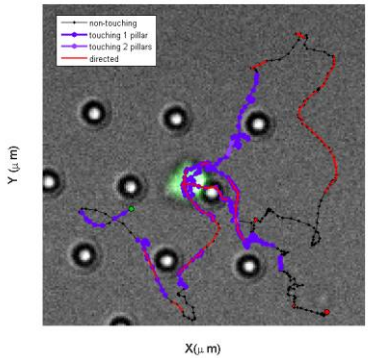
New Journal of Physics 16, 075012 (2014)

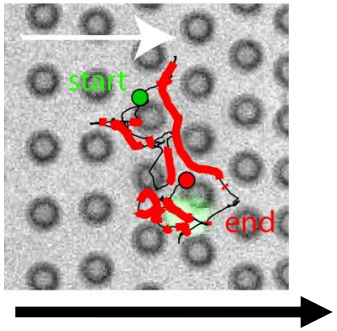
Annu. Rev. Cond. Matter Phys. 1:257 (2010)

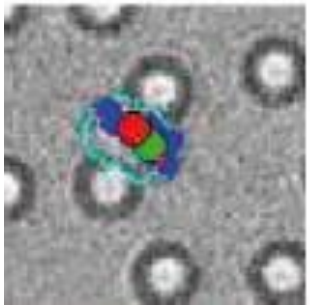
Surface Structures as Cell Attractors

Directed Migration | Quasi-Random Migr. | Drift Migration | Cell Trapping







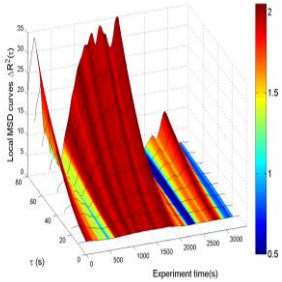


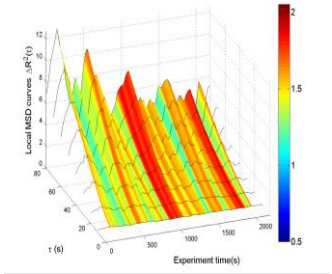
Straight motion

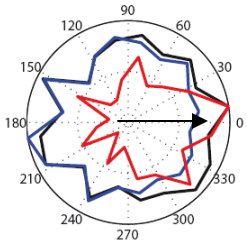
Localized in structures

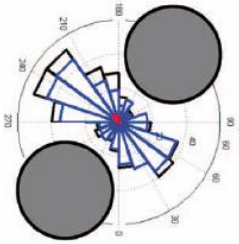
Due to density gradient

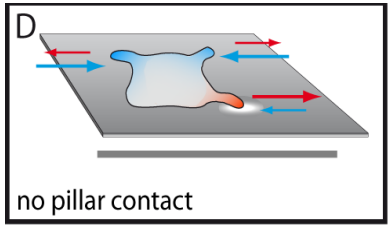
Confinement

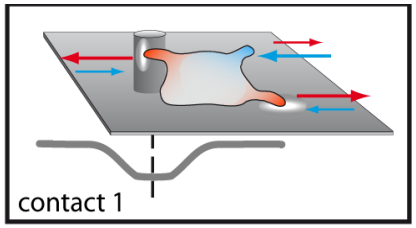


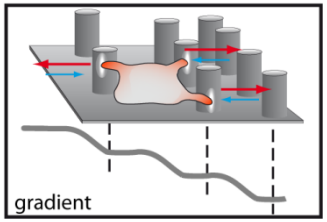


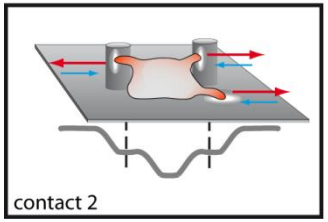












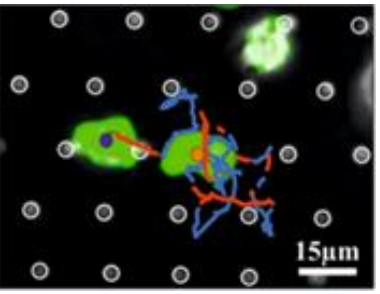
Arcizet, Meier, Sackmann, Rädler, Heinrich, *Phys. Rev. Lett. 101, 248103* (2008)

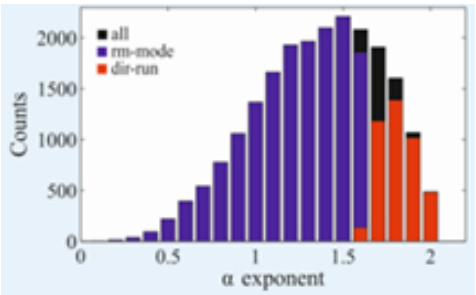
Sackmann, Keber, Heinrich, *Annu. Rev. Cond. Matter Phys. 1*:257 (2010)

Arcizet, Capito, Gorelashvili, Leonhardt, Youssef, Rappl, Heinrich, *Soft Matter*8(5):1473 (2012)

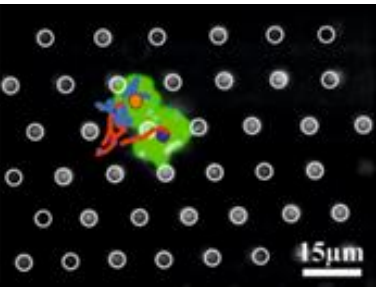
M. Gorelashvili, M. Emmert, K. F. Hodeck and D. Heinrich, New Journal of Physics 16, 075012 (2014)

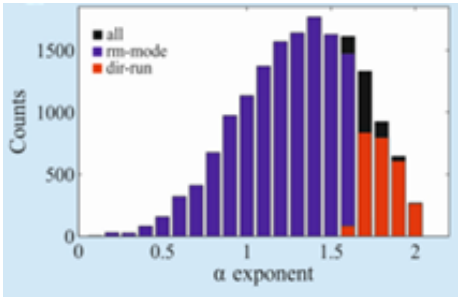
Topographic Guidance Effects



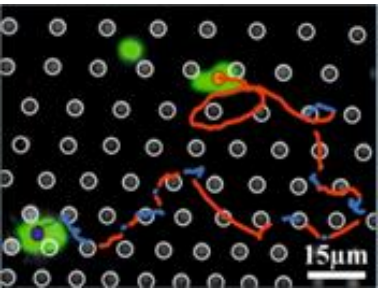


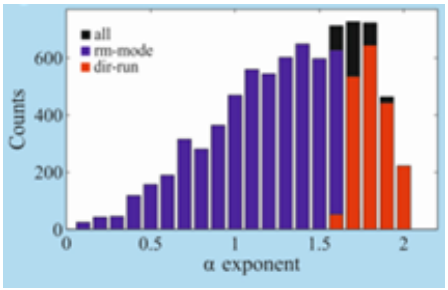
Low



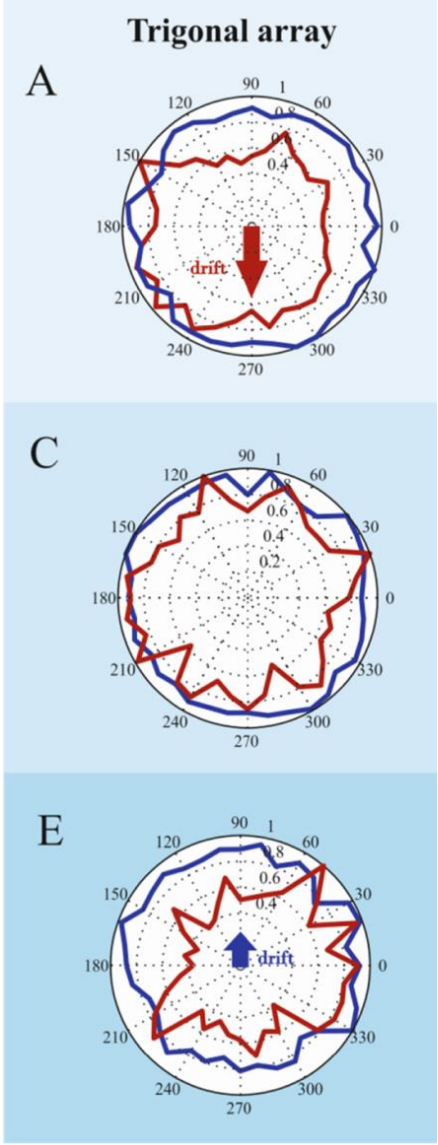


Pillar Density





High



Change in cell population for $\Delta t = 12$ h

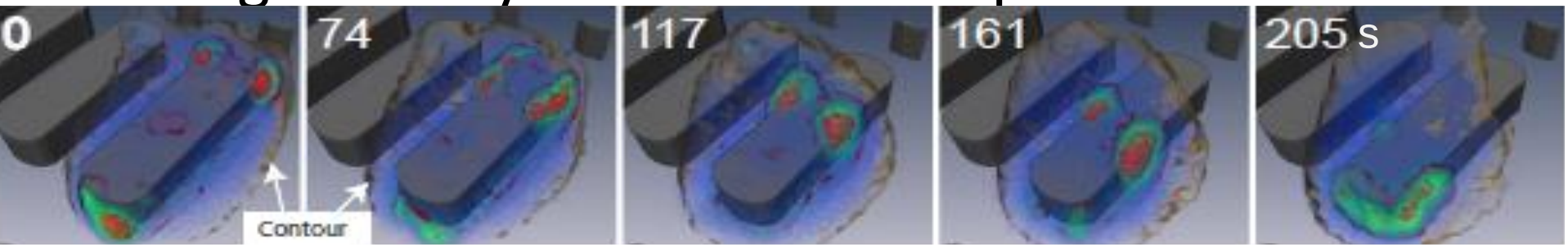
- 3 %

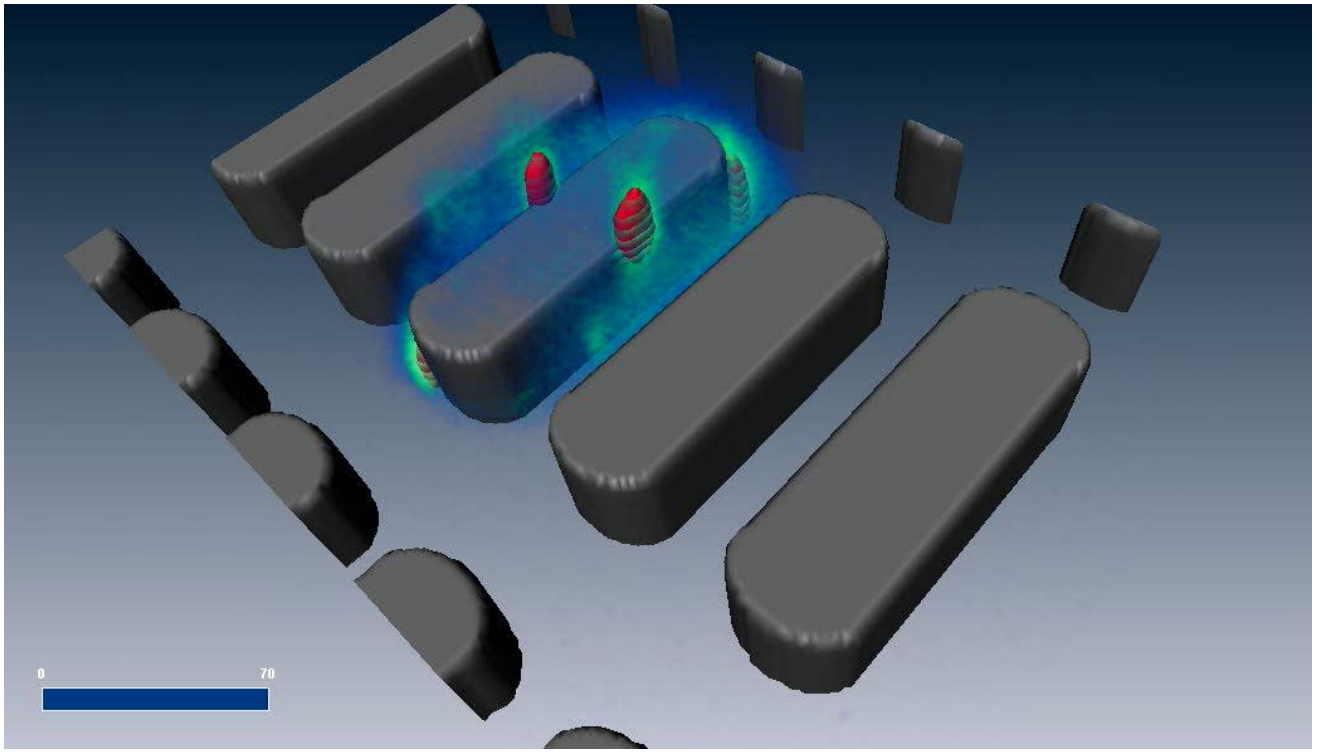
+ 12 %

- 33 %

Arcizet, Capito, Gorelashvili, Leonhardt, Youssef, Rappl, Heinrich, *Soft Matter*8(5):1473 (2012)
M. Gorelashvili, M. Emmert, K. F. Hodeck and D. Heinrich, New Journal of Physics 16, 075012 (2014)

Cell Migration by Actin Waves in quasi-3D Structures





5 µm

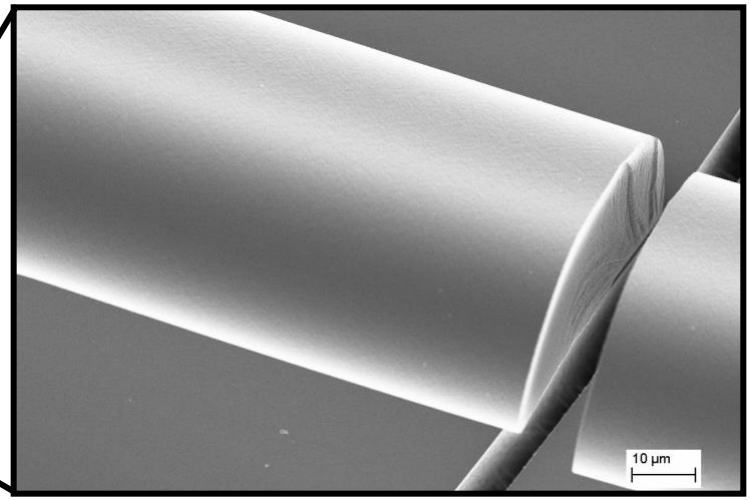
Red	Green	Blue	Grey
Actin Polymerization	Actin Depolymerization	Cell Outline	Surface Structure

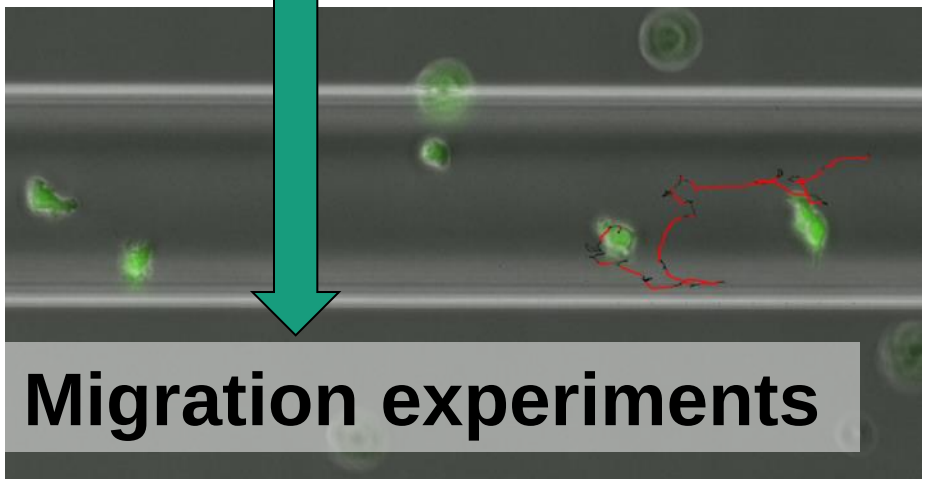
E. Sackmann, F. Keber and D. Heinrich, Annu. Rev. Cond. Mat Phys. 2010

Analysis of living cell interaction with silica fibers

3D Scaffold

2D Model



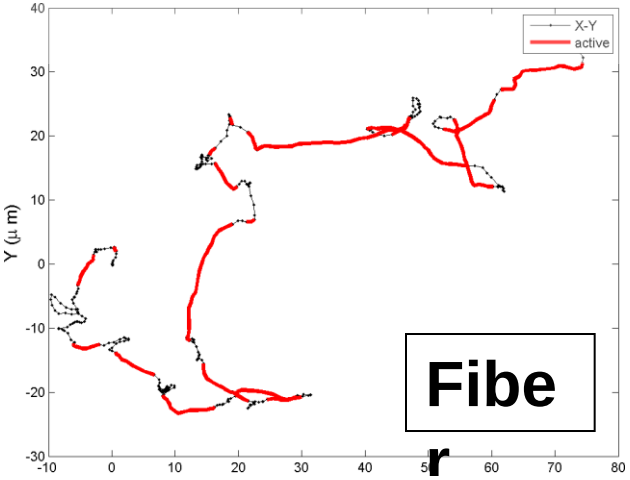


Migration experiments

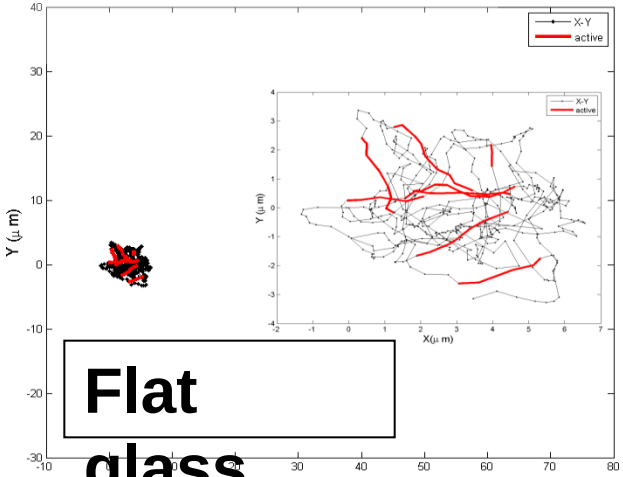
Analysis of amoeboid cell migration on fibers vs. flat glass surfaces

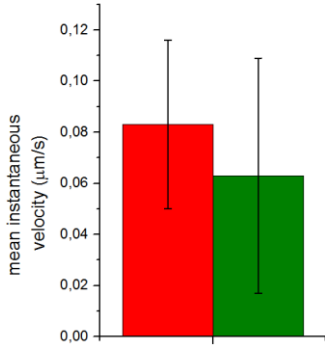
Fraunhofer ISC

M. Emmert, P. Witzel, M. Rothenburger-Glaubitt, and D. Heinrich, RSC Advances, 2017

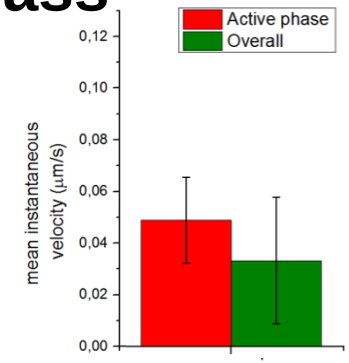


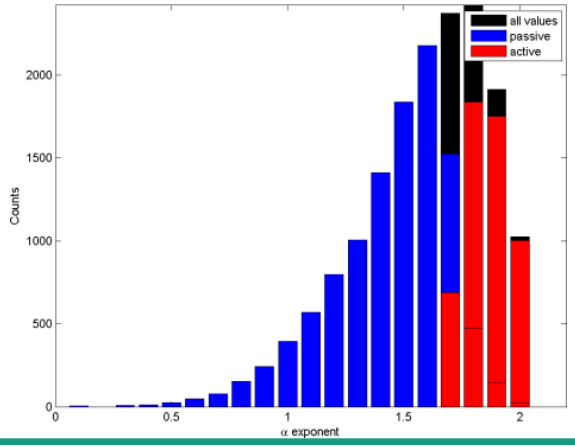
Typical trajectory



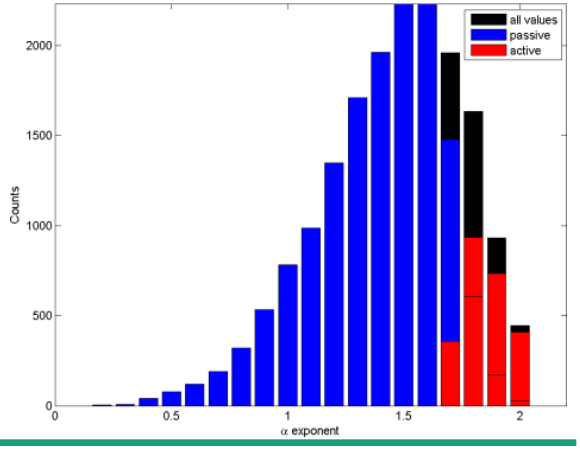


Mean instantaneous velocity





α exponent



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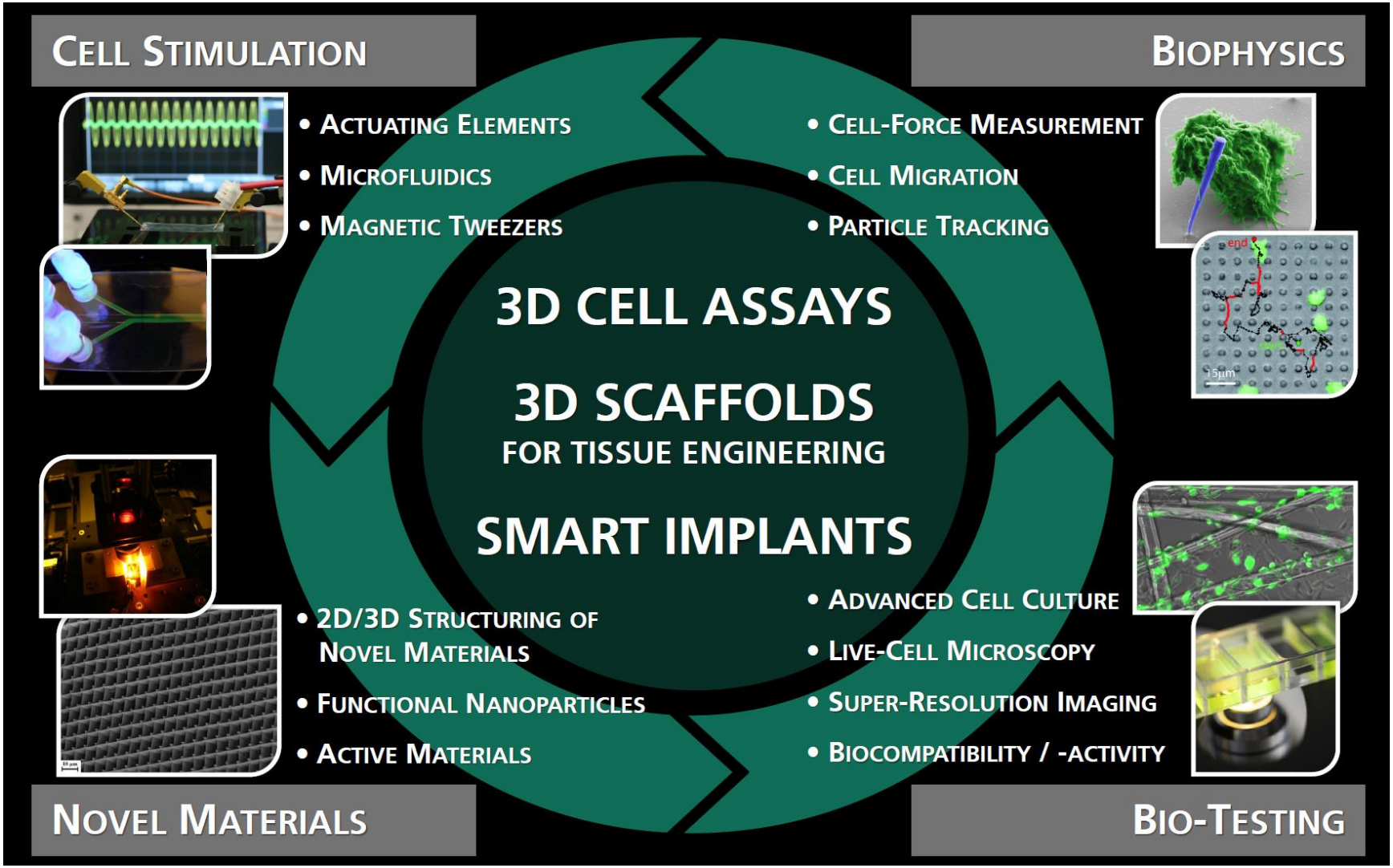
M. Emmert, P. Witzel, M. Rothenburger-Glaubitt, and D. Heinrich, RSC Advances, 2017

Heinrich Lab - Expertise



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