

## P20 – Optical Force Based Investigations of Cell Mechanical Concepts During Phagocytosis

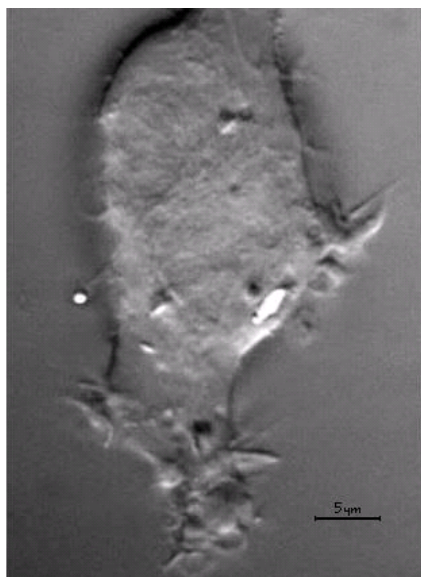
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A central mechanism of the mammalian immune system is the internalization of bacteria by macrophages during phagocytosis. However, the mechanical properties of phagocytosis are largely unknown, in particular when mediated by cellular tentacles like filopodia. We used optical tweezers-based microscopy to investigate different mechanical concepts of the cell to take up 1  $\mu\text{m}$  beads, which serve as synthetic bacteria. The motion of an optically trapped bead was tracked interferometrically in 3D with nanometer precision at a microsecond timescale. On the one hand, the measurement of the thermal bead fluctuations during the



Differential interference contrast (DIC) image of a macrophage during phagocytosis of a 1  $\mu\text{m}$  latex bead.

binding to the cell membrane enabled the observation of individual receptor-ligand bond formation. On the other hand, the measurement of the mean bead displacements allowed determining retraction forces of filopodia at various retraction speeds. We measured F-actin dependent 36-nanometer steps inside living cells during filopodia retraction likely belonging to actin-based molecular motors [1]. Steps remained clearly visible even at force regimes clearly beyond the stall force of a single myosin motor. This seems to indicate a kind of inter-motor coupling, a phenomenon which we try to explain by a stochastic multi-state model. We want to combine the position detection of the bead with fluorescence microscopy techniques to investigate the reorganization of the cell during filopodial retraction and the underlying concepts of phagocytosis.

[1] Kress, H., E.H.K. Stelzer, D. Holzer, F. Buss, G. Griffiths, and A. Rohrbach: "Filopodia act as phagocytic tentacles and pull with discrete steps and a load-dependent velocity", Proc. Nat. Acad. Sci., Vol.104, 2007, 11633–11638



# Optical force based investigations of cell mechanical concepts during phagocytosis

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## Abstract

Filopodia are thin cell surface protrusions containing bundles of actin filaments. The behavior of these cellular tentacles is the object of our research. Macrophages internalize bacteria during phagocytosis, which is a central mechanism in the immune system. Still, only little is known about the mechanical properties of phagocytosis, in particular when mediated by filopodia. We used optical tweezers-based microscopy to investigate mechanical concepts of cell to take up 1 μm beads, which serve as synthetic bacteria. The motion of an optically trapped bead was tracked interferometrically in 3D with nanometer precision at a microsecond timescale. On the other hand, the measurement of the mean bead displacements allowed determining retraction forces of filopodia at various retraction speeds. We measured F-actin dependent 36-nanometer steps inside living cells during filopodia retraction likely belonging to actin-based molecular motors [2]. Steps remained clearly visible even at force regimes clearly beyond the stall force of a single myosin motor. This seems to indicate a kind of inter-motor coupling, a phenomenon which we try to explain by a stochastic multi-state model.

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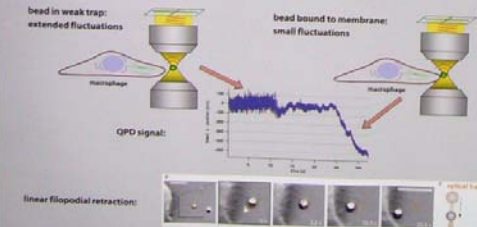
## Cell protrusions act as tentacles

Membrane protrusions (filopodia and ruffles) act as tentacles and pull bound beads towards the cell



Macrophage cells are key components of the innate mammalian immune system. They internalize pathogens to degrade them intracellularly. Here we show that macrophage cell membrane protrusions act as tentacles and pull bound objects towards the cell prior to phagocytosis.

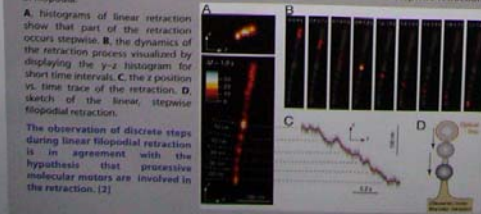
### Experimental procedure



a. An IgG-coated bead in the optical trap (orange circle in the bottom picture row) is moved towards a filopodium of a 7774 macrophage. Upon binding, the filopodium (arrowhead) retracts and pulls the bead towards the cell to initiate phagocytic uptake. c. Sketch of the linear retraction respectively. [1]

## 3D retraction kinetics

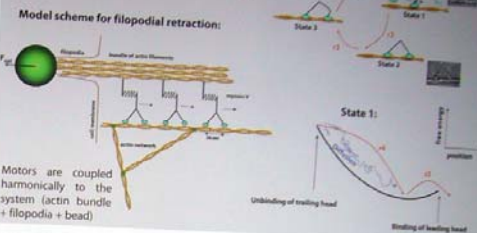
The kinetics of the retraction events is measured in 3D with nanometer precision. 2D projections (x- and y-plane) of 3D bead position histograms reveal the dynamics of linear stepwise retraction of filopodia.



The observation of discrete steps during linear filopodial retraction is in agreement with the hypothesis that processive molecular motors are involved in the retraction. [2]

## Model for filopodial retraction by cooperative molecular motors

Motorproteins (myosin V) are described with a stochastic multi state model. The main step (state 1) is rate limited by diffusion in a harmonic potential given by the inner molecular strain. [4]



## Simulation of filopodial retraction

With Brownian dynamic (BD) simulations we describe cooperative movement of molecular motors, which are coupled harmonically to the system of actin bundle, filopodia and bead. Solving the Langevin equation leads to a stochastic motor displacement  $\Delta x_{i,t}$  (i motor index):

$$\Delta x_{i,t} = \frac{-\gamma V_i + F_{i,t} + \epsilon_i \sqrt{2k_B T \gamma} \xi_i}{\gamma} \Delta t$$

and a displacement of the system (filopodia + bead)  $\Delta x_t$ :

$$\Delta x_t = \frac{F_{i,t} + \sum_{j=1}^N F_{j,t} + \epsilon_t \sqrt{2k_B T \gamma} \xi_t}{\gamma} \Delta t$$

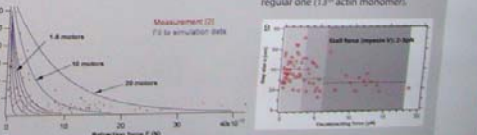


### Force-velocity curve for linear filopodial retraction:

Speed of linear filopodial retraction as a function of the counteracting force applied by the optical tweezers (red points) [2]. BD simulation data for different number of involved motors in blue.

### Future features of model / simulation:

Number of involved motors controlled by force dependent unbinding. Stepsize variation due to binding to actin monomers adjoining to the regular one (13<sup>th</sup> actin monomer).



## Summary and Conclusions

A photonic force microscope allows loading cell tentacles with small particles and measuring their retraction behavior in 3D. For the linear movement we observe a step-wise retraction of the bead attached to the cell tentacle likely belonging to 36 nm steps of processive myosin V motors walking along actin filaments. We expect a coordinated operation of several molecular motors at higher loading forces and reduced retraction velocity. The coordinated movement can be described by a stochastic multi state model for molecular motor harmonically coupled to the actin bundle in the filopodia. BD simulations reveal that stepsizes can be observed for various motor couplings, which depend on the motor's neck stiffness. A qualitative agreement of the velocity force  $v/F$  relationship with experimental data can be achieved even in the case of a constant number of involved motors.

## References

- [1] Kress, Stelzer, Griffiths, and Rohrbach, "Radiation pressure control in optical traps and application to phagocytic membrane binding" Phys. Rev. E, 71, 061927 (2005)
- [2] Kress, Steiner, Holzer, Buss, Griffiths, and Rohrbach, "Filopodia act as phagocytic tentacles and pull with discrete steps and a load-dependent velocity". PNAS. 104, 25, 11633-11638 (2007).
- [3] Rohrbach, "Trapping and tracking a local probe with a photonic force microscope". RevSciInstrum. 75, 2197 (2004).
- [4] Walker, Burgess, Sellers, Wang, Hammer III, Itrick and Knight, "Two-headed binding of a processive myosin to F-actin. Nature. 405, 804-807 (2003).

