P9 – Multiple Facets of Neuronal Growth: Different Implementations of SFM

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A prerequisite for an understanding of the mechanisms of nerve regeneration and neuronal development is the understanding of the principles of force generation in growth cones – the mechanical and chemical sensor of a neuron. In our working group we try to approach this issue from a variety of different aspects, combining experimental and theoretical studies of actin networks, membrane fluctuations and focal adhesion sites. Two other aspects which will be presented here are the direct measurement of forces exerted by the cells, and the neuron's response to mechanical and chemical stimuli.After we succeeded in measuring stall forces and forces of the retrograde actin flow and the cell body with an SFM in fast-moving fish keratocyte cells we want to transpose this method to growth cones. The SFM cantilever is kept at constant height and force while the growth cone is growing against the bead which is glued at the tip, and the lateral deflection is recorded. Because forces and velocities of neurons are very small and the resulting experiment times are long, the SFM setup requires additional stabilization. We want to use a dual-beam optical trap setup to monitor potential drift of sample and cantilever to ensure a stable position of the cantilever, with respect to the sample, over very long times.

Another facet is chemo- and durotaxis of neurons. We use soft materials with locally modifies Young's modulus, measured with SFM, and local protein coatings to induce highly polarized cell growth. The aim is to learn how such a stimulus is reflected on the molecular level and how this knowledge can be transferred to whole-cell behavior, e.g. cell differentiation, preconceiving that cells are highly adaptable systems. First results of experiments with such materials modified by a new treatment technique are presented here, along with the description of the stabilized SFM setup.

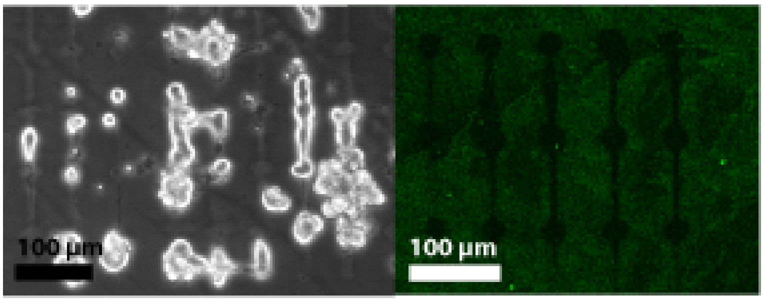


Figure 1 NG 108-15 cell extending on the soft stripes of a polyacryl amide gel *(left)* although there is no surface protein coating (*right*, fluorescent stain of laminin)

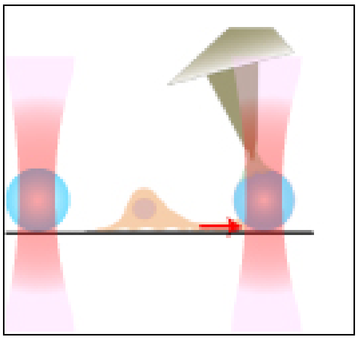
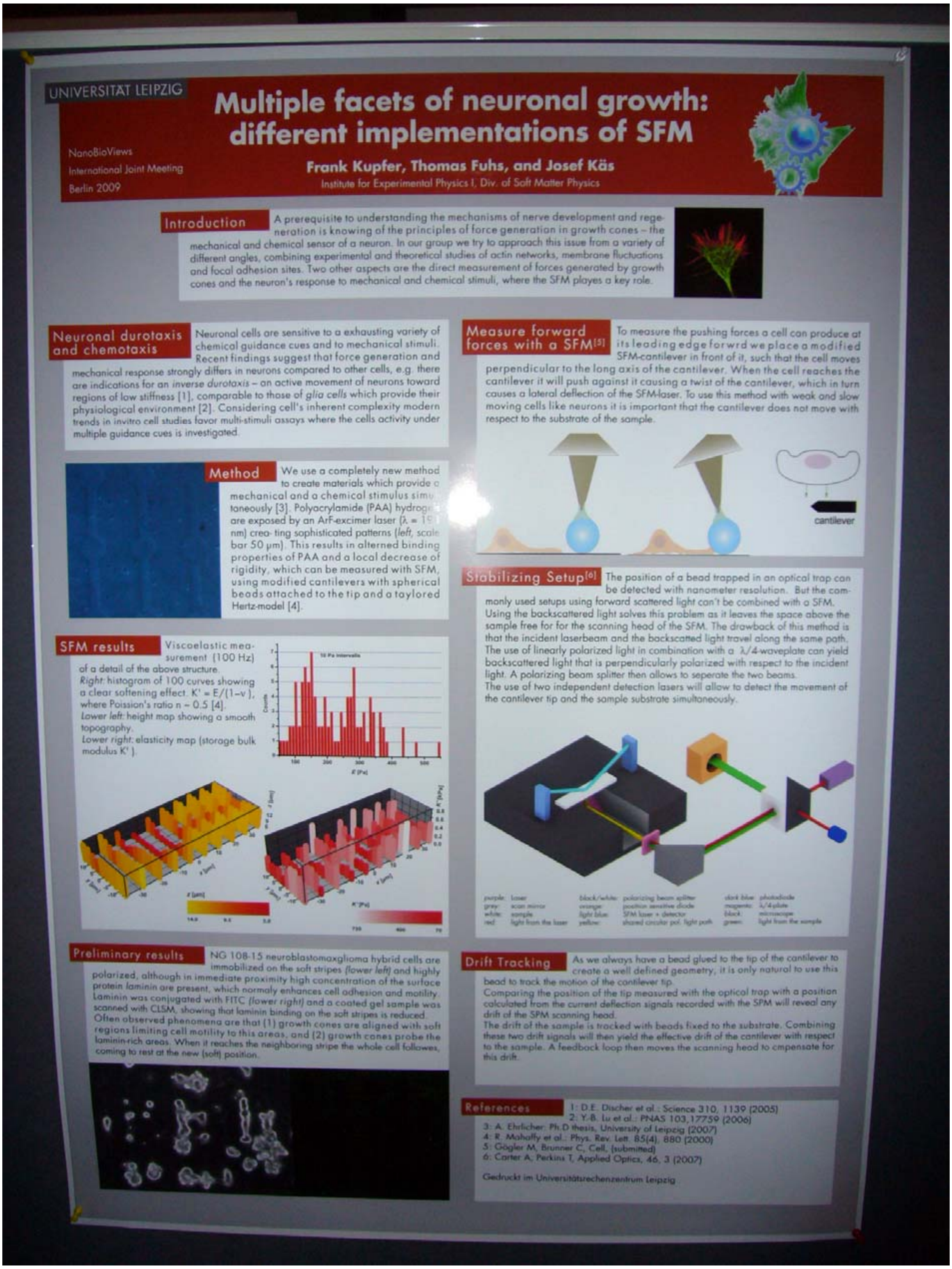


Figure 2 Schematics of force measurement with dual-beam optical trap stabilizing sample (left bead) and cantilever (right bead)



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NanoBioViews
International Joint Meeting
Berlin 2009

Multiple facets of neuronal growth: different implementations of SFM

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Introduction

A prerequisite to understanding the mechanisms of nerve development and regeneration is knowing of the principles of force generation in growth cones – the mechanical and chemical sensor of a neuron. In our group we try to approach this issue from a variety of different angles, combining experimental and theoretical studies of actin networks, membrane fluctuations and focal adhesion sites. Two other aspects are the direct measurement of forces generated by growth cones and the neuron's response to mechanical and chemical stimuli, where the SFM playes a key role.

Neuronal durotaxis and chemotaxis

Neuronal cells are sensitive to a exhausting variety of chemical guidance cues and to mechanical stimuli. Recent findings suggest that force generation and mechanical response strongly differs in neurons compared to other cells, e.g. there are indications for an *inverse durotaxis* – an active movement of neurons toward regions of low stiffness [1], comparable to those of *glia cells* which provide their physiological environment [2]. Considering cell's inherent complexity modern trends in invitro cell studies favor multi-stimuli assays where the cells activity under multiple guidance cues is investigated.

Method

We use a completely new method to create materials which provide a mechanical and a chemical stimulus simultaneously [3]. Polyacrylamide (PAA) hydrog are exposed by an ArF-excimer laser (λ = 19 nm) crea- ting sophisticated patterns (*left*, scale bar 50 µm). This results in alterned binding properties of PAA and a local decrease of rigidity, which can be measured with SFM, using modified cantilevers with spherical beads attached to the tip and a taylored Hertz-model [4].

SFM results

Viscoelastic measurement (100 Hz) of a detail of the above structure.
Right: histogram of 100 curves showing a clear softening effect. $K' = E/(1-\nu)$, where Poission's ratio n ~ 0.5 [4].
Lower left: height map showing a smooth topography.
Lower right: elasticity map (storage bulk modulus K').

Preliminary results

NG 108-15 neuroblastomaxglioma hybrid cells are immobilized on the soft stripes *(lower left)* and highly polarized, although in immediate proximity high concentration of the surface protein *laminin* are present, which normaly enhances cell adhesion and motility. Laminin was conjugated with FITC *(lower right)* and a coated gel sample was scanned with CLSM, showing that laminin binding on the soft stripes is reduced.
Often observed phenomena are that (1) growth cones are aligned with soft regions limiting cell motility to this areas, and (2) growth cones probe the laminin-rich areas. When it reaches the neighboring stripe the whole cell followes, coming to rest at the new (soft) position.

Measure forward forces with a SFM[5]

To measure the pushing forces a cell can produce at its leading edge forwrd we place a modified SFM-cantilever in front of it, such that the cell moves perpendicular to the long axis of the cantilever. When the cell reaches the cantilever it will push against it causing a twist of the cantilever, which in turn causes a lateral deflection of the SFM-laser. To use this method with weak and slow moving cells like neurons it is important that the cantilever does not move with respect to the substrate of the sample.

cantilever

Stabilizing Setup[6]

The position of a bead trapped in an optical trap can be detected with nanometer resolution. But the commonly used setups using forward scattered light can't be combined with a SFM. Using the backscattered light solves this problem as it leaves the space above the sample free for for the scanning head of the SFM. The drawback of this method is that the incident laserbeam and the backscatted light travel along the same path. The use of linearly polarized light in combination with a λ/4-waveplate can yield backscattered light that is perpendicularly polarized with respect to the incident light. A polarizing beam splitter then allows to seperate the two beams.
The use of two independent detection lasers will allow to detect the movement of the cantilever tip and the sample substrate simultaneously.

purple:	laser	black/white:	polarizing beam splitter	dark blue:	photodiode
grey:	scan mirror	orange:	position sensitive diode	magenta:	λ/4-plate
white:	sample	light blue:	SFM laser + detector	black:	microscope
red:	light from the laser	yellow:	shared circular pol. light path	green:	light from the sample

Drift Tracking

As we always have a bead glued to the tip of the cantilever to create a well defined geometry, it is only natural to use this bead to track the motion of the cantilever tip.
Comparing the position of the tip measured with the optical trap with a position calculated from the current deflection signals recorded with the SPM will reveal any drift of the SPM scanning head.
The drift of the sample is tracked with beads fixed to the substrate. Combining these two drift signals will then yield the effective drift of the cantilever with respect to the sample. A feedback loop then moves the scanning head to cmpensate for this drift.

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Gedruckt im Universitätsrechenzentrum Leipzig