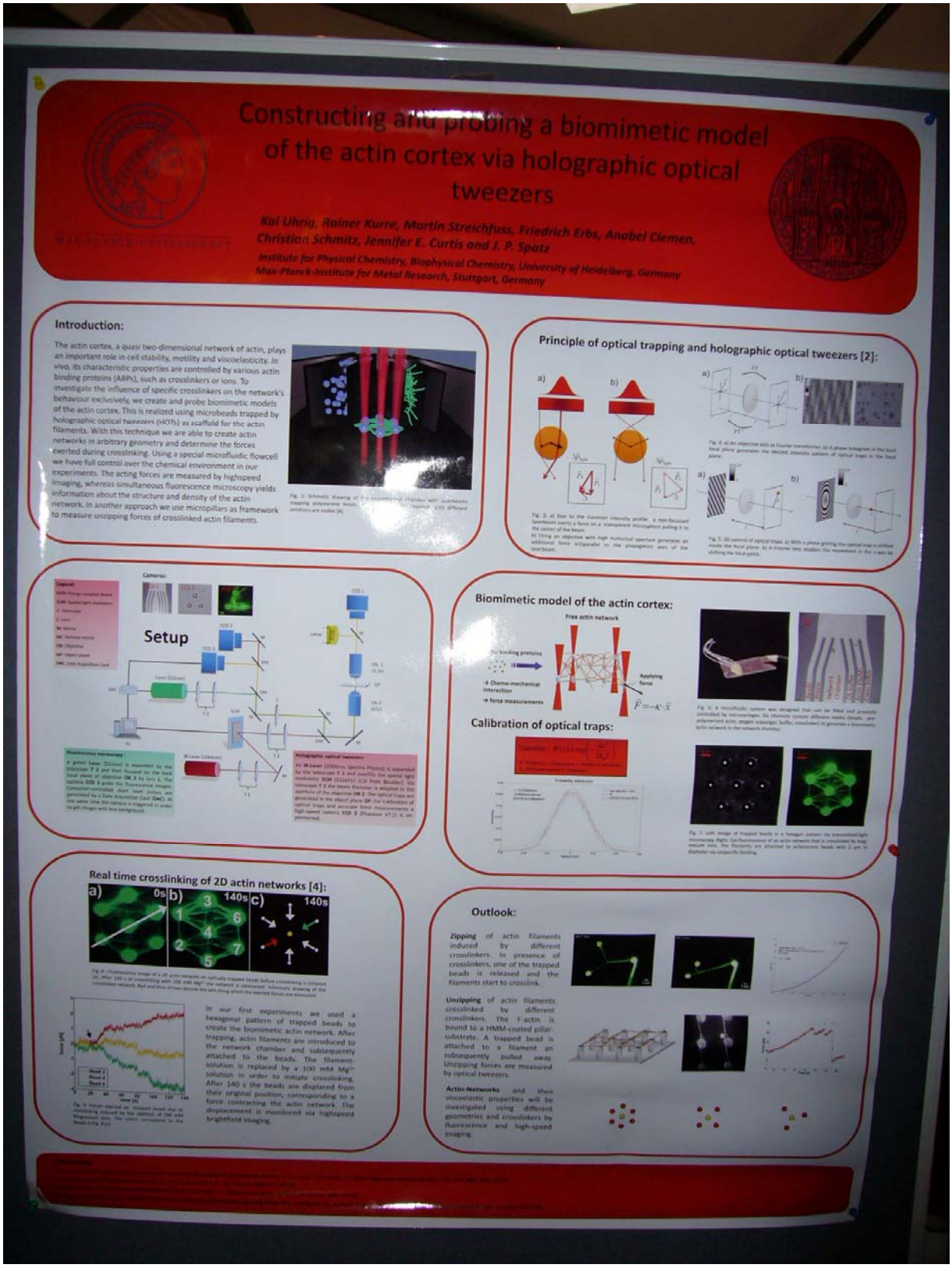
P2 – Constructing and Probing a Biomimetic Model of the Actin Cortex via Holographic Optical Tweezers

Martin Streichfuss

Max Planck Institute for Metals Research
Heidelberg, Germany

The actin cortex, a quasi two-dimensional network of actin, plays an important role in cell stability, motility and viscoelasticity. *In vivo*, its characteristic properties are controlled by various actin binding proteins (ABPs), such as crosslinkers or ions. To investigate the influence of a specific crosslinker on the network's behaviour exclusively we create and probe biomimetic models of the actin cortex. This is realized using microbeads trapped by holographic optical tweezers (HOTs) as scaffold for the actin filaments. With this technique we are able to create actin networks in arbitrary geometry and determine the forces exerted by different crosslinkers. Using a special microfluidic flowcell we have full control over the chemical environment in our experiments. The acting forces are measured by highspeed imaging, whereas simultaneous fluorescence microscopy yields information about the structure and density of the actin network. In another approach we use micropillars as framework and measure unzipping forces of crosslinked actin filaments.


Constructing and probing a biomimetic model of the actin cortex via holographic optical tweezers
Kai Uhrig, Rainer Kurre, Martin Streichfuss, Friedrich Erbs, Anabel Clemen, Christian Schmitz, Jennifer E. Curtis and J. P. Spatz
Institute for Physical Chemistry, Biophysical Chemistry, University of Heidelberg, Germany
Max-Planck-Institute for Metal Research, Stuttgart, Germany
Introduction:
The actin cortex, a quasi two-dimensional network of actin, plays an important role in cell stability, motility and viscoelasticity. In vivo, its characteristic properties are controlled by various actin binding proteins (ABPs), such as crosslinkers or ions. To investigate the influence of specific crosslinkers on the network's behaviour exclusively, we create and probe biomimetic models of the actin cortex. This is realized using microbeads trapped by holographic optical tweezers (HOTs) as scaffold for the actin filaments. With this technique we are able to create actin networks in arbitrary geometry and determine the forces exerted during crosslinking. Using a special microfluidic flowcell we have full control over the chemical environment in our experiments. The acting forces are measured by highspeed imaging, whereas simultaneous fluorescence microscopy yields information about the structure and density of the actin network. In another approach we use micropillars as framework to measure unzipping forces of crosslinked actin filaments.
Principle of optical trapping and holographic optical tweezers [2]:
Setup
Biomimetic model of the actin cortex:
Free actin network
Calibration of optical traps:
Real time crosslinking of 2D actin networks [4]:
0s
140s
140s
In our first experiments we used a hexagonal pattern of trapped beads to create the biomimetic actin network. After trapping, actin filaments are introduced to the network chamber and subsequently attached to the beads. The filament solution is replaced by a 100 mM Mg^{2+} solution in order to initiate crosslinking. After 140 s the beads are displaced from their original position, corresponding to a force contracting the actin network. The displacement is monitored via highspeed brightfield imaging.
Outlook:
Zipping of actin filaments induced by different crosslinkers. In presence of crosslinkers, one of the trapped beads is released and the filaments start to crosslink.
Unzipping of actin filaments crosslinked by different crosslinkers. The F-actin is bound to a HMM-coated pillar-substrate. A trapped bead is attached to a filament an subsequently pulled away. Unzipping forces are measured by optical tweezers.
Actin-Networks and their viscoelastic properties will be investigated using different geometries and crosslinkers by fluorescence and high-speed imaging.