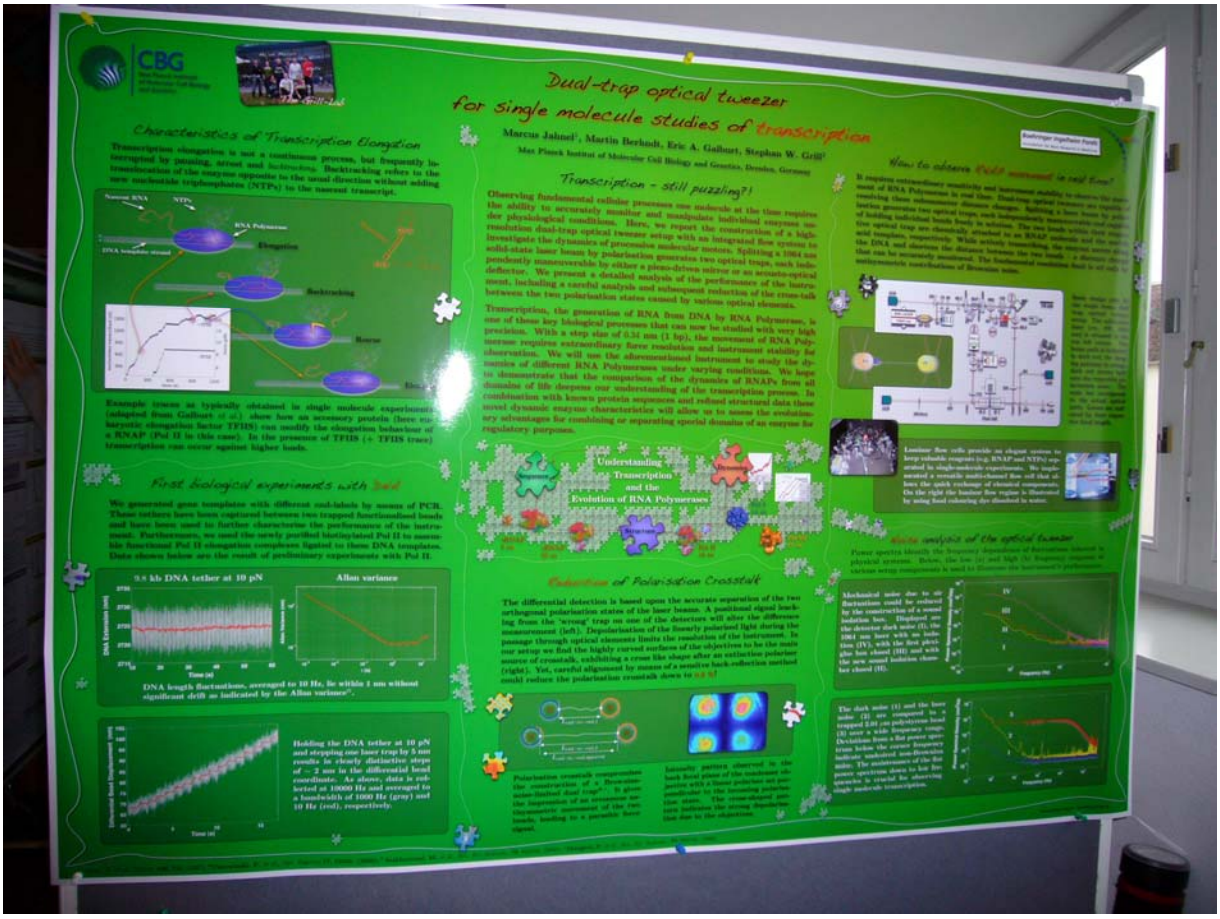
P14 – Dual-Trap Optical Tweezer for Single Molecule Studies of Transcription

Marcus Jahnel, Martin Behrndt, Stephan W. Grill

Max Planck Institute of Molecular Cell Biology and Genetics,
Pfotenhauerstr. 108, 01307 Dresden, Germany,
jahnel@mpi-cbg.de, September 29, 2009

Observing fundamental cellular processes one molecule at the time requires the ability to accurately monitor and manipulate individual enzymes under physiological conditions. Here, we report the construction of a high-resolution dual-trap optical tweezer setup with an integrated flow system to investigate the dynamics of processive molecular motors. Splitting a 1064 nm solid-state laser beam by polarisation generates two optical traps, each independently maneuverable by either a piezo-driven mirror or an acousto-optical deflector. We present a detailed analysis of the performance of the instrument, including a careful analysis and subsequent reduction of the cross-talk between the two polarisation states caused by various optical elements.

Transcription, the generation of RNA from DNA by RNA Polymerase, is one of these key biological processes that can now be studied with very high precision. With a step size of 0.34 nm (1 bp), the movement of RNA Polymerase requires extraordinary force resolution and instrument stability for observation. We will use the aforementioned instrument to study the dynamics of different RNA Polymerases under varying conditions. We hope to ultimately demonstrate that the comparison of the dynamics of RNAPs from all domains of life deepens our understanding of the transcription process and allows us to assess the evolutionary advantages for combining or separating special domains of an enzyme for regulatory purposes.


CBG
Dual-trap optical tweezer
for single molecule studies of transcription
Characteristics of Transcription Elongation
Transcription - still puzzling?!
How to observe
First biological experiments with
Understanding
Transcription
and the
Evolution of RNA Polymerases
Reduction of Polarisation Crosstalk
Allan variance