

## **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,  
Pfortenhauerstr. 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.



## Dual-trap optical tweezers for single molecule studies of transcription

Marcin Jahnke<sup>1</sup>, Martin Berthold, Eric A. Gallart, Stephan W. Grill<sup>1</sup>  
Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany



### Characteristics of Transcription Elongation

Transcription elongation is not a continuous process, but frequently interrupted by pausing, arrest and backtracking. Backtracking refers to the movement of the core in opposite to the usual direction without adding new nucleotides triphosphates (NTPs) to the nascent transcript.



Example traces as typically obtained by single molecule experiments (adapted from Gallart et al.) show how an accessory protein (here eukaryotic elongation factor TFIID) can modify the elongation behaviour of a RNAP (Pol II in this case). In the presence of TFIID (+ TFIID trace) transcription can occur against higher loads.

### First biological experiments with dual traps

We generated gene complexes with different nucleotides by means of PCR. These factors have been captured between two trapped functional beads and have been used to further characterize the performance of the instrument. Furthermore, we used the newly purified histone-like Pol II to measure functional Pol II elongation complexes ligated to these DNA constructs. These traces below are the result of preliminary experiments with Pol II.



0.8 kb DNA tether at 10 pN

DNA length fluctuations, averaged to 10 Hz, fit within 1 nm without significant drift as indicated by the Allan variance.



Allan variance



Holding the DNA tether at 10 pN and stepping one laser trap by 5 nm results in clearly distinguishable steps of ~2 nm in the differential bead coordinates. An example data is published at 10000 Hz and averaged to a bandwidth of 1000 Hz (grey) and 10 Hz (red), respectively.

### Transcription - still puzzling!

Observing fundamental cellular processes *in situ* 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 tweezers setup with an integrated flow system to investigate the dynamics of prokaryotic molecular motors. Splitting a 1000 nm solid-state laser beam by polarisation generates two optical traps, each independently manoeuvrable 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 those 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 demonstrate that the comparison of the dynamics of RNAPs from all several dynamic enzymes characteristics will allow us to assess the evolutionary advantages for combining or separating spatial elements of an enzyme for regulatory purposes.

### Understanding Transcription and the Evolution of RNA Polymerases

The differential detection is based upon the accurate separation of the two orthogonal polarisation states of the laser beams. A positional signal resulting from the 'wrong' trap on one of the detectors will alter the difference measurement (dF). Deposition of the laser's projection light during the passage through optical elements limits the resolution of the instrument. In our setup we find the highly curved surfaces of the objectives to be the main source of cross-talk, exhibiting a cross-like shape after a collection polariser source of cross-talk, exhibiting a cross-like shape after a collection polariser source (right). Yet, careful alignment for means of a sensitive back collection method could reduce the polarisation cross-talk down to 0.2 %.



Polarisation cross-talk originates from the combination of a theoretical cross-talk 'dead zone'. It gives the combination of all possible cross-talk components, leading to a possible three spots.

Isolated pattern observed in the back focal plane of the instrument, together with a linear polariser set perpendicular to the incoming polarisation state. The cross-shaped pattern indicates the strong birefringence due to the objectives.

### How to observe 'back movement' in real time?

It requires extraordinary accuracy and instrument stability to observe the movement of RNA Polymerase in real time. Dual-trap optical tweezers are capable of holding individual beads steady. Additional 1 laser beam by polarisation generates two optical traps, each independently manoeuvrable and capable of holding individual beads steady. The two traps, which hold the DNA and the motor, respectively. While actively measuring, the detector system (DVS) and detector the distance between the two beads. A detector system can be accurately measured. The fundamental resolution limit is set only by the measurement uncertainties of the detector system.



Low-force force traps provide an elegant system to keep individual components (e.g. RNAP and NTPs) separated in single-molecule experiments. We implemented a sensitive differential method that not only allows the high-resolution of distance measurements, but also the high-resolution of force measurements. On the right the low-force force traps are illustrated by using dual-colouring, also illustrated by a trace.

### Single analysis of the optical tweezers

Force spectra identify the frequency spectrum of the movement induced by physical contact. Below, the low (L) and high (H) frequency spectra of the back-trap spring components, in red (L) and blue (H) respectively, are shown.



Mechanical noise due to the laser fluctuations could be reduced by the use of a single trap. The noise spectrum of a single trap is shown in red (L) and blue (H) respectively. The noise spectrum of a single trap is shown in red (L) and blue (H) respectively. The noise spectrum of a single trap is shown in red (L) and blue (H) respectively.

The dark noise (L) and the force spectra (H) are compared to a 100 nm wide dynamic range. The noise spectrum of a single trap is shown in red (L) and blue (H) respectively. The noise spectrum of a single trap is shown in red (L) and blue (H) respectively.