Automated measurements with the JPK RampDesigner™ Software

Many interdisciplinary researchers from various fields of science have argued that only the combination of biological, biochemical, and biophysical approaches can deliver a broader insight into the fundamental processes driving biological systems. As a consequence, an increasing number of research groups are including mechanical aspects, material properties, and the influence of forces in their studies of bio-molecular interactions and cellular dynamics. A significant amount of time and effort has been invested, not only in characterizing the mechanical properties of biological samples, but also in designing new instruments and experiments that are suitable for actually determining these properties. Extremely small scales and fragmentary knowledge of the local material mix, e.g. in different compartments of living cells, are only two of the obstacles to overcome.

Soft biological matter and macro-molecules are among the most complex materials in terms of composition, mechanical properties and dynamic behavior. Thus, characterizing biological samples requires sophisticated experimental procedures and data analysis routines. The mechanical properties of cells, for example, are not fully determined by methods that measure responses to a single linear external perturbation alone. The dynamic properties of force generating proteins, e.g. motor proteins, are also difficult to characterize due to the stochastic nature of their movements.

RampDesigner™

Applying and measuring advanced force patterns with sophisticated setups like atomic force microscopes (AFMs) or optical tweezers (OT) can be time consuming and often require more than basic skills in software programming. The purpose of JPK's RampDesigner™ software module is to facilitate the process of creating and modifying multi-step schemes of force application to biological samples. The integration of feedback modes for applying and maintaining well-defined forces makes JPK's RampDesigner™ a versatile toolbox for biological, medical and biophysical research. It's modular structure and user-friendly graphical interface enables researchers to set up and edit advanced measurement cycles with just a few mouse clicks.

Modular Structure

Experimental sequences in the RampDesigner™ are composed of smaller modules, so called segments. These can be linear movements of the trap or sample, oscillations, or pausing segments where data is recorded from a stationary optical trap. More sophisticated segments include force ramps (linear movement to build up a pre-defined force) and force clamps (dynamic trap or sample repositioning to maintain a defined force). Also applications like the tracking of motor proteins are covered by specific segments.

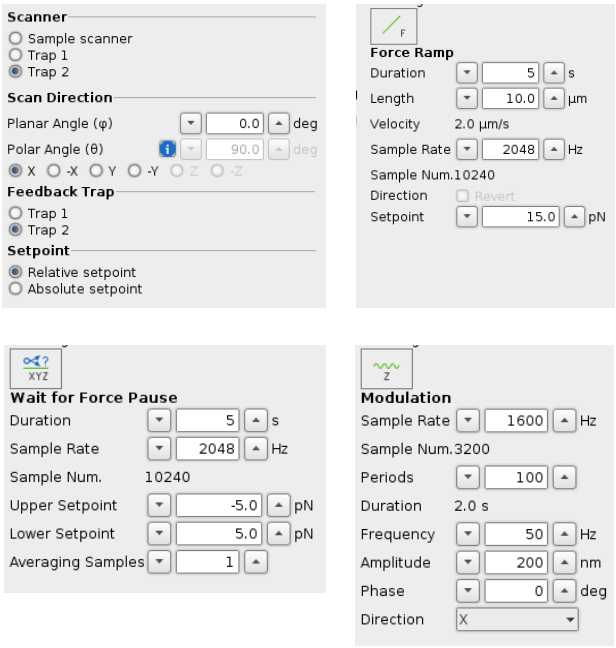

Figure 1 *Selection of available segments*

In general, segments are used to define the movement of one of the system's scanners, the two optical traps and the piezo driven sample scanner. Global settings (selected scanner, direction of movement, relative/absolute setpoint) are valid throughout the measurement. Segments can be selected from a menu and added to the sequence of actions at any position. Depending on the type of movement, a number of settings can be defined, e.g. the distance and speed of a linear movement, or the frequency, amplitude and phase of a sine oscillation.

Segment Types

In a **"Force Ramp"** segment, a setpoint force (amount, direction) and the scanner speed can be defined. The selected scanner moves in the given direction until the setpoint force has been reached. The setpoint can be defined either as an absolute value or relative to the force measured at the beginning of the segment.
Once the measured force matches the setpoint, the measurement ends or the next segment is executed. Setpoint values can be carried over to subsequent segments or be reset.

A **"Force Clamp"** segment typically follows the force ramp. During the force clamp, the feedback controlled scanner updates its position in order to maintain the setpoint force. The only other parameter is the duration of the clamp. An optional, built-in safety feature ("Bead saver") reduces the risk of losing the particle when the system is unable to maintain the setpoint force, e.g. when the particle detaches from the sample. If the force signal exceeds or falls below a certain threshold (as typically happens when the scanner moves very fast and the particle is displaced too far from the trap), the segment is aborted.

The so called **"Wait for force"** segment is similar to the force ramp, except it only has a stationary scanner. It is used for active systems (e.g. cells or motor proteins) that can build up forces by themselves. The trapped probe particle is held in a constant position until the external force acting on it reaches a pre-defined value. Two setpoint forces can be defined, one above and one below the force value at the beginning of the segment. Therefore, it is not necessary to know the exact orientation of the expected external force before the measurement.
In addition to the above segments, "**Position Ramps**" (linear movements with defined speed, distance, direction), "**Modulation**" segments (scanner oscillations with defined amplitude, frequency and phase), and "**Pause**" segments are available.
Figure 1 shows some of the segment types and the available settings.

Soft Matter Rheology

Cellular matter is visco-elastic as it is basically composed of polymeric networks and fluids. The mechanical response thus strongly depends on the time scale and load rate (i.e. force-time relation) of the stimulation. The capability of living cells to actively adapt their mechanical properties to external cues or their functional status, adds another dimension of time-dependent characteristics.
Various experimental schemes have been developed to fully characterize the complex mechanical properties of living cells or their components. Tang and Ngan employed a so called rate-jump method [1] while other approaches investigate mechanical responses to oscillating external forces over a broad range of frequencies [2]. A review of methods can be found in the publication by Pullarkat et al. [3].
With the RampDesigner™ software, a series of periodic forces with increasing frequency or at different pre-loaded states can easily be generated. Analysis of the recorded data in JPK's DataProcessing software is also based on segments, so that different mathematical operations can be applied to the whole dataset, segment by segment. This is particularly convenient in the case of micro-rheological measurements, where the material response is investigated at different oscillation frequencies (corresponding to different segments of the measurement). A key characteristic of these measurements is the phase difference between force application and absolute particle movement, as it is directly related to the viscous and elastic contributions to the material response (see also our Application Note "Micro-rheology of cells and soft matter with the

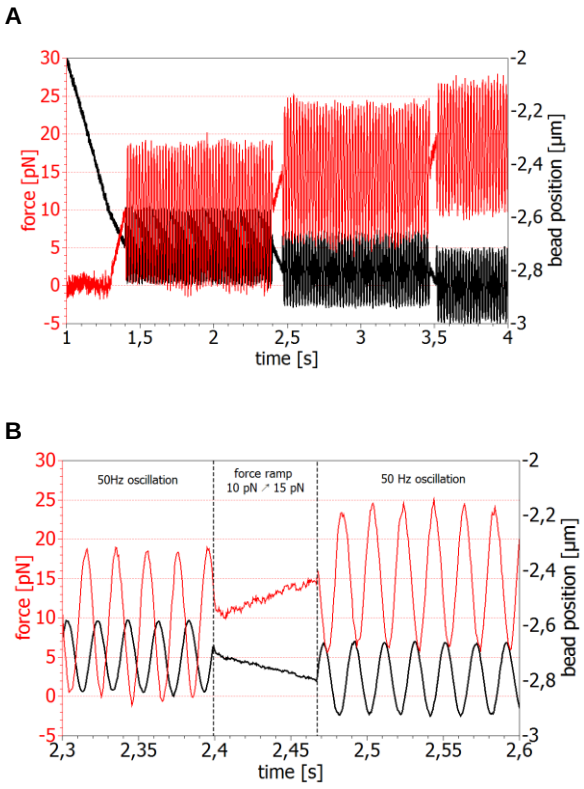

Figure 2 *Application of periodic forces to a red blood cell.* ***A*** *The measurement is a combination of force ramps and oscillations. The trap moves the bead towards the cell until a force of 10pN is reached. This is followed by an oscillation (A=200nm, f=50Hz), an increase of the force by 5pN, another oscillation and so on.* ***B*** *Zoomed in on the time axis. The transition between an oscillation around 10pN and one around 15pN is shown. The trap moves the bead further towards the cell (black curve) until the next setpoint of 15pN is reached (red curve).*

NanoTracker™" which can be downloaded from www.jpk.com). Figure 2 shows the application of periodic forces to a red blood cell at different pre-loaded forces (10, 15, and 20 pN). The corresponding RampDesigner sequence would be ForceRamp (10 pN)-Oscillation-ForceRamp (15 pN)-Oscillation-ForceRamp (20 pN)-Oscillation. Data from the transition between two oscillation segments is shown in Figure 2B: The trap moves closer to the cell until the next force setpoint is reached. Then, the oscillation segment starts.

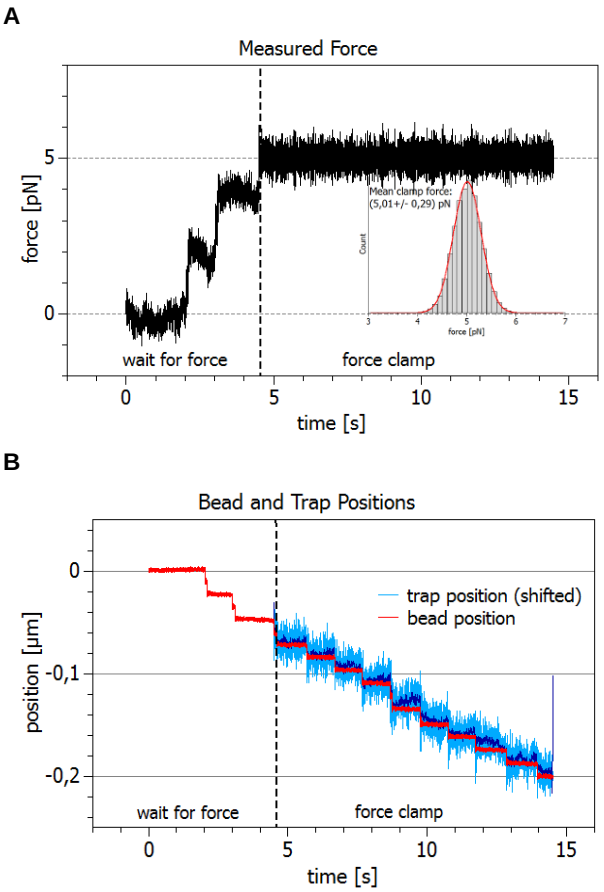

Figure 3 *Wait for force and force clamp segment.* ***A*** *The trapped particle is moved away from the trap center by an external force. As soon as the setpoint (5pN) is reached, the trap starts to follow the particle's movement in order to keep the force constant (black curve). The mean of the measured force values during clamping is 5.01+/-0.29 pN.* ***B*** *The traces of the bead (red) and the trap (blue) show how the system tracks the particle's movement in real time. The trap position was shifted to overlay the particle position. In the raw data, there is a constant displacement between trap and bead position corresponding to the setpoint force.*

Active Systems

The mechanisms of force generation in biological matter are manifold. Many of them, particularly on small scales, are driven by biased stochastic interactions between

different components, e.g. the heads of motor proteins and their substrates. Through regulative processes, the cell influences the probability of certain events taking place, and thus controls molecular dynamics. In the case of motor proteins, mechanical load, the availability of chemical energy, and other factors can influence the speed and direction of movement [4]. For these and similar situations, JPK developed the "Wait for force" segment, a dual-setpoint force clamp. Two different setpoints can be defined above and below the starting value. The scanner then remains in a stationary state until one of the setpoints has been reached. Figure 3 shows an example of a simulated[1] motor protein that walks in steps of approx. 12.5 nm and increases the force on the attached bead until the pre-defined value (5 pN) is reached during the third step. After this, the trap position is automatically updated to follow the protein movement under a constant force load. As shown in Figure 3B, the trap follows the particle (and thus the motor protein) movement while the force is kept constant with high accuracy Figure 3A).

DNA under force

The mechanical properties of DNA and how these are influenced by interactions with other molecules has been in the focus of numerous studies. It is well established that stretching a double-stranded DNA (ds-DNA) beyond its contour length L_c results in the (partially reversible) disintegration of the molecule. During this so-called overstretching, individual bonds in the macromolecule break and the contour length of the damaged molecule increases.

The required forces are in the range of 60-70pN [5]. The experimental setup in optical traps usually comprises of two beads with different surface functionalizations, which bind to the two ends of the ds DNA. The beads are optically trapped and the force is recoded while the molecule is elongated. Applying a force clamp with a setpoint near the overstretching transition creates a situation where the dsDNA is likely to disrupt in multiple steps. As long as the molecule's end-to-end length L_{e-e} (which corresponds to the distance between the two particle surfaces) is smaller than L_c, the force-distance curve can be theoretically described with the wormlike-chain (WLC) model for polymer dynamics [6]. As soon as

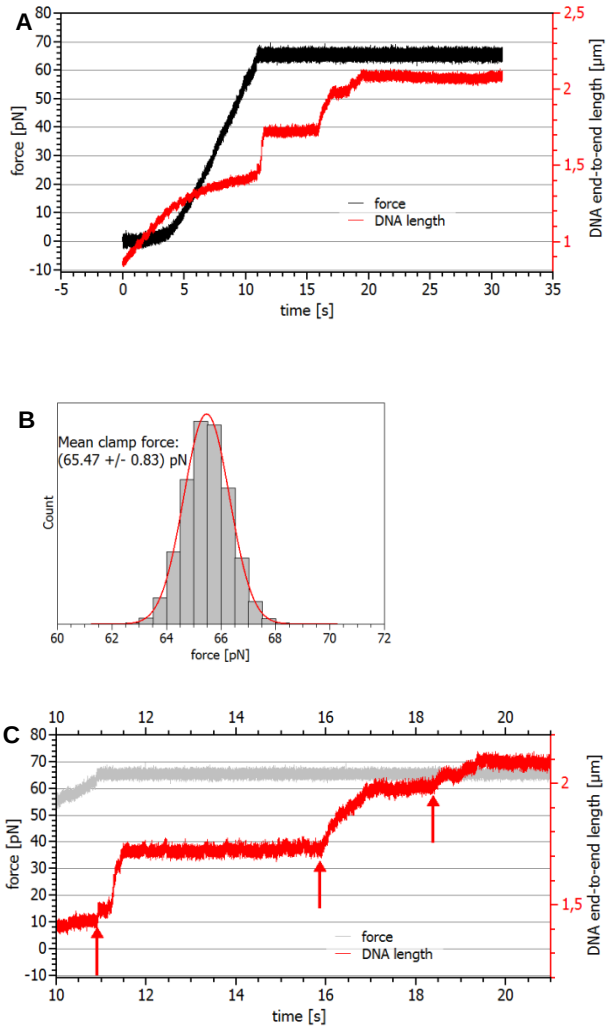

Figure 4 A *Force-extension curve of a dsDNA molecule in a force clamp. It is stretched until the setpoint of 65pN is reached, then the force is kept constant (black curve). Since this is near the over-stretching transition of the molecule, it starts to disintegrate and it's length increases (red curve).* ***B*** *Mean value and standard deviation of the measured force values during the clamp segment.* ***C*** *Detail of the step-wise elongation of dsDNA under constant force. Each event corresponds to the breaking of internal bonds in the DNA.*

[1] A 2µm polystyrene bead was attached to a glass surface and moved with a piezo sample scanner to mimic the motor protein movement.

$L_{e-e} \geq L_c$, the dsDNA elongates stepwise under constant force. Figure 4 shows the whole process of stretching and holding the dsDNA at 65pN force. The feedback loop compensates the elongation of the molecule that accompanies the break-up of internal structures by repositioning one of the traps. In the red curve ($L_{e-e}(t)$), these events are clearly visible (red arrows). Depending on the buffer properties (pH, T, …) and the presence of DNA-interacting molecules, the behavior of the DNA molecule changes in terms of variations in stiffness and overstretching force.

Conclusion

With the latest version of the RampDesigner™ software module for the NanoTracker™, JPK provides an extensive toolbox for performing complex force applications and measurements in biological samples. The mechanical behavior of single molecules, viscoelastic materials and whole cells can be investigated with extremely flexible force patterns. The required effort to set up and modify an experimental sequence is reduced to a minimum, leaving more time for the actual acquisition and analysis of data.

Literature

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