Optical tweezers measurements of the DNA conformational dynamics at the single-molecule level

DNA Breathing
Application note

2018

LUMICKS

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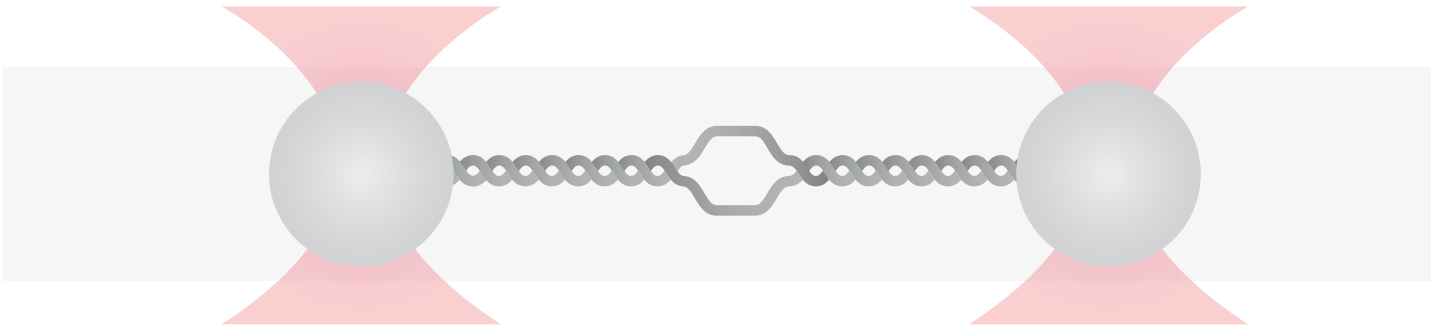
Application Note



1 A schematic representation of a DNA breathing experiment. A dsDNA molecule is tethered between two optically-trapped microspheres and held at a certain tension, while a DNA bubble is spontaneously formed.

Real-time observation of DNA "breathing"

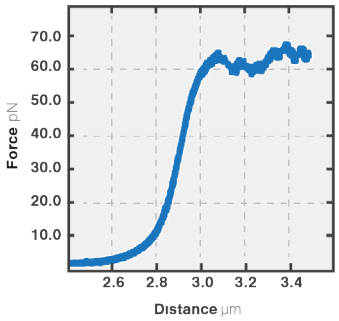
In order to unlock and correctly transfer the genetic information of the cell, contained within the DNA, a myriad of essential biological interactions is required. The progression of such DNA processing reactions (e.g. replication, transcription or repair) is not only guided by the interaction with specific enzymes, but also by the dynamics of the physical properties of the DNA itself. These can either facilitate or halt specific interactions at different time points. From this structural perspective, DNA is a highly malleable molecule, susceptible to for example bending or breathing.

DNA breathing, also known as fraying, describes the local conformational transitions occurring within double-stranded DNA (dsDNA), when base pairs break spontaneously without requiring any external energy source. This phenomenon results in local sections, known as DNA bubbles, where dsDNA is temporarily converted to single-stranded DNA (ssDNA). These DNA bubbles can subsequently act as anchor points for processing proteins to interact.

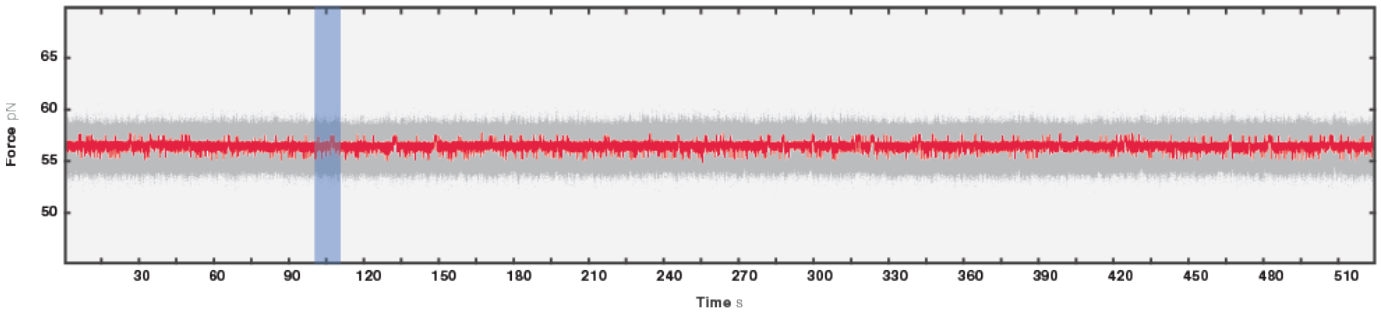
Due to the transient and spontaneous nature of this process, the study of real-time DNA breathing transitions at the single-molecule level requires the use of highly sensitive technologies. Using the high-resolution optical tweezers and microfluidics developed by LUMICKS it is possible to study DNA breathing transitions by measuring length changes down to the sub-nm level.

Additionally, using the integrated laminar flow microfluidics allows for an automated in situ formation of DNA tethers between optically-trapped microspheres (Figure 1). The latter enables the molecule to access different DNA breathing transitions and titrate associated effects such as sequence stability or frequency of bubble formation.

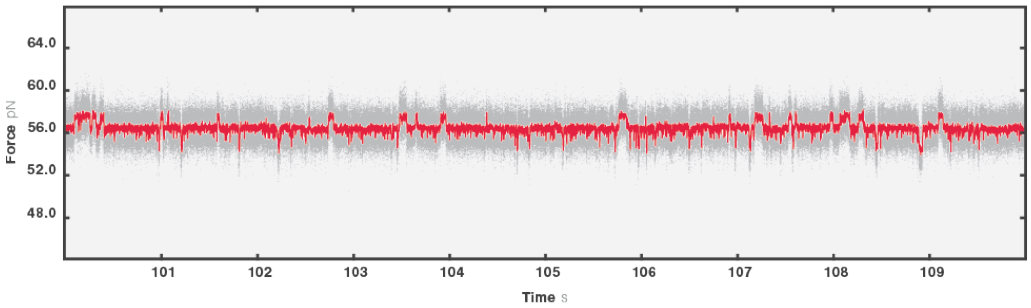
In the experiment performed in LUMICKS' laboratories, a single dsDNA molecule (8.4 kbp) was stretched to a tension of approximately 65 pN in a low-salt buffer (10 mM TRIS), while recording its corresponding force-extension curve (Figure 2). The low salt concentration ensures an increased repulsion between the two DNA strands due to the negative charges along the DNA phosphate backbone, thereby facilitating the formation of bubbles at a high DNA breathing frequency when the tension was maintained close to the overstretching plateau (i.e. 58 pN). By maintaining the end-to-end distance constant over time, we observed fluctuations in the recorded force signal, revealing DNA breathing. Under these conditions, we were able to monitor this phenomenon for over 500 s, because of the low drift of LUMICKS' optical tweezers (Figure 3a).



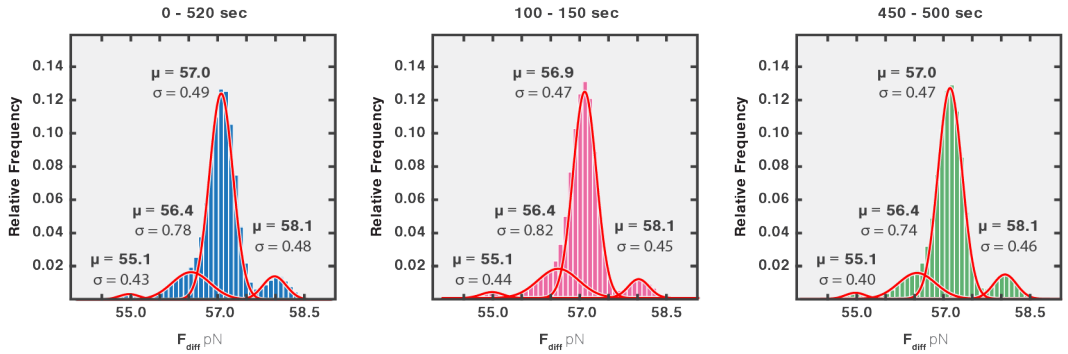
2 Force extension curve corresponding to a single dsDNA molecule (8.4 kbp) contained in a low-salt buffer (10 mM TRIS). The dsDNA molecule was held between two optically-trapped streptavidin-coated polystyrene beads, via the biotins decorating both 3' and 5'-ends of a single strand. At a tension of approximately 65 pN, the molecule accessed the overstretching transition, where unpeeling was observed as a series of bursts.



3a Force trace recorded over 520 seconds corresponding to a single dsDNA molecule (8.4 kbp) held at a constant distance using optically-trapped polystyrene beads (Ø= 0.8 µm). The trap stiffness was set to 500 pN/µm. Data was recorded at 50 kHz (gray) and decimated to 1000 Hz (red).



3b 10 seconds fragment of the trace shown within the purple inset in figure **3a**



3c Histogram of the force values collected for the full trace.

3d Histogram of the force values collected for the 50 s fragment between 100-150 s.

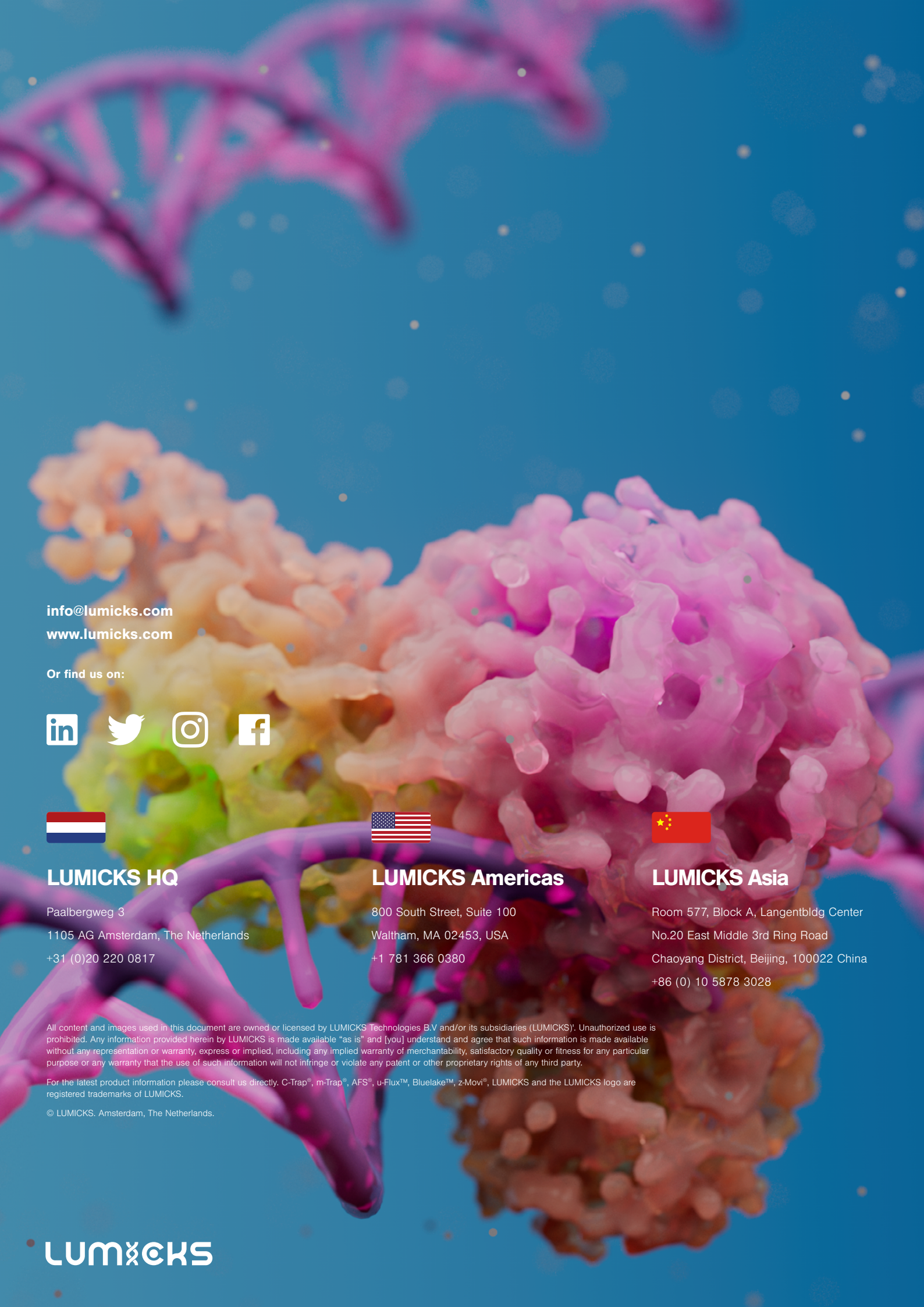
3e Histogram of the force values collected for the 50 s fragment between 450-500 s.

3c-e Histograms of the force values collected for the full trace (**3c**) and for values collected during two different fragments of 50 seconds (**3d** and **3e**). The mean and sigma values are reported for each peak obtained from a Gaussian fit.

When looking at a 10 s inset of the complete trace, fast transitions between multiple states are clearly visible (Figure 3b). In fact, when we performed histogram analysis over the full trace (Figure 3c), up to 4 states displaying DNA breathing corresponding to up to 60 base pairs were resolved.

Additionally, when looking at the histograms of two 50 s sections distributed along the main trace (Figure 3d and 3e), identical features can be seen. This not only indicates that the experiment was performed without altering the transition kinetics, but also shows the ultra-high stability of the instrument.

Using LUMICKS' high resolution optical tweezers, small and transient DNA conformational changes can be monitored in real-time with an unprecedented stability, providing scientists with valuable information on the number and frequency of various conformational stages.



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