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Tuning the Music: Acoustic Force Spectroscopy (AFS) 2.0

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ABSTRACT

AFS is a recently introduced high-throughput single-molecule technique that allows studying structural and mechanochemical properties of many biomolecules in parallel. To further improve the method, we developed a modelling tool to optimize the layer thicknesses, and a calibration method to experimentally validate the modelled force profiles. After optimization, we are able to apply 350 pN on 4.5 μm polystyrene beads, without the use of an amplifier, at the coverslip side of the AFS chip. Furthermore, we present the use of a transparent piezo to generate the acoustic force and we show that AFS can be combined with high-NA oil or water-immersion objectives. With this set of developments AFS will be applicable to a broad range of single-molecule experiments.

1. Introduction

The ability to mechanically manipulate single biomolecules is leading to insights in fundamental cell processes [1–4]. Single-molecule force-spectroscopy techniques are commonly used to study, for example denaturation of biomolecules [5], the binding, unbinding and folding of proteins [6] and the replication and repair of nucleic acids [7]. However, single-molecule instruments can be rather complex and often have a low experimental throughput [2,8]. Moreover, the dynamics of single molecules are intrinsically stochastic, meaning that, to probe the heterogeneous behaviour, many independent measurements should be performed [2,9].

Acoustic Force Spectroscopy (AFS) is a recently introduced single-molecule technique that distinguishes itself by a high experimental throughput, a wide range of forces that can be applied and an unmatched range of force loading rates [10] (Fig. 1A). In its original implementation the method has several drawbacks: it makes use of an opaque piezo-element to generate the acoustic force, the force profile is not constant over the fluid layer and it is not possible to apply significant forces close to the coverslip side.

Here we present innovative solutions to address these limitations. The use of a transparent piezo-element allows trans-illumination of the sample, which increases the tracking accuracy of beads. We optimize the layer thicknesses of the system to generate a strong force at the bottom surface and quantify the modelled forces by direct measurement of the force profile. Furthermore, we show that AFS is also compatible with high numerical aperture (NA) water or oil-immersion objectives. In addition, we made the force gradient that is present in the fluidic cell flatter by using multiple frequencies at the same time. A flatter force profile improves the accuracy of the force application because of the diminished force variations when a measured construct changes length. Finally, we demonstrate that is it possible to turn AFS into a distance clamp.

2. Material and methods

2.1. AFS-chip properties

An AFS chip consists of two glass layers with a fluid channel in between and a piezo element on top. The flow cells are custom fabricated by LUMICKS B.V. Flow cells of 2 different dimensions are used in this report. Flow cell 1 has layer thicknesses of 1000, 100 and 175 μm and flow cell 2 has 616, 84 and 175 μm for the matching, fluid and capping layers, respectively (Fig. 1). The total tilt of the flow cells is less than 1 milliradian and the surface roughness is less than 1 nm (LUMICKS specifications). Both flow cells are glued (using Permatex, 40150A) either to a 500 μm piezo or to a 200 μm piezo resulting in 4 different types of AFS-chips.

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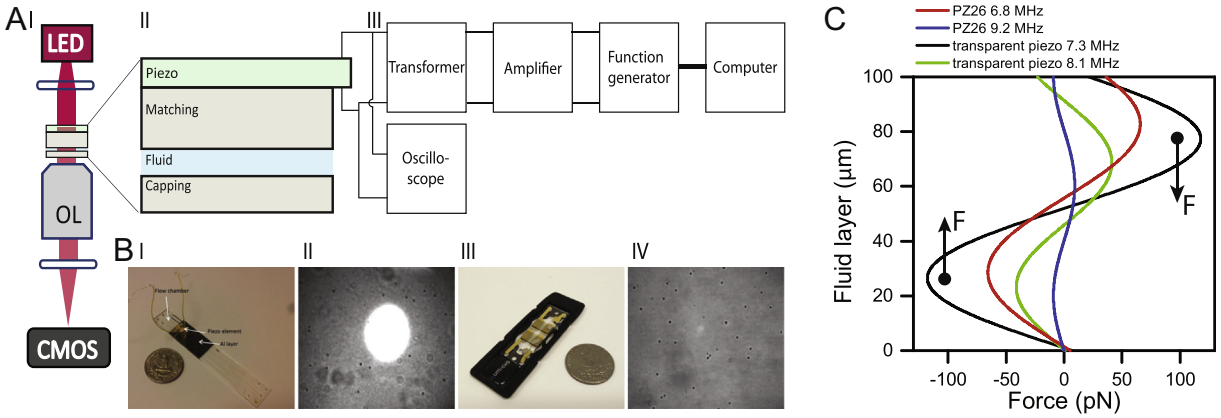

Fig. 1. Illustration of the AFS setup. (A, I) The AFS chip is imaged using an inverted microscope with objective lens (OL), a digital CMOS camera and LED light source (455 nm). (A, II) The flow cell consists of two glass plates with a fluid chamber in between. A transparent piezo element is attached to the upper glass slide. Using an overhang the piezo is electronically connected. (A, III) Diagram hardware configuration, the function generator is controlled by the computer via USB, while it is connected in series with an amplifier, a transformer and to the piezo element. An oscilloscope is connected to measure the voltage over the piezo. (B) A picture of the AFS chip (I, III) and a digital camera image (II, IV) are shown for the non-transparent and transparent piezo chip, respectively. (C) Modelled force profiles for transparent and opaque piezo element, in both cases two resonance frequencies are displayed with a force directed toward and away from the top surface (indicated with the arrows acting on beads). Forces are calculated for a 4.5-µm-diameter polystyrene microsphere with an input power of 0.1-W.

2.2. Electrical connection of the chip

The piezo is driven by a function generator (Siglent, SDG830) at frequencies ranging from 1 to 30 MHz via a power RF-amplifier (SCD, ARS 2_30_30, 50-Ω impedance, 10-W max. output power). A transformer is used to match the output electrical impedance of the amplifier and the function generator to the electrical impedance of the layered resonator. The electrical impedance of each layered resonator configuration used is measured and a custom transformer is made to match the impedance (Supplementary Fig. 1). The peak-to-peak voltage over the piezo was recorded with an oscilloscope (Tektronix, TDS1002). The function generator is connected to the computer via USB and controlled with a LabVIEW interface (Fig. 1B, III).

2.3. Imaging the sample

An inverted bright-field microscope (Nikon eclipse Ti-E) was equipped with a 1 megapixel 60 Hz frame-rate CMOS camera (Thorlabs, DCC3240M) read out via USB by a computer. A collimated LED (Thorlabs, M455L3-C5) was coupled into a condenser lens (Nikon, LWD 0.52) to illuminate the sample. The sample was then imaged with a 40× or a 10× microscope objective (Nikon, CFI Plan Fluor 40× and CFI Plan Fluor 10×) in combination with a 0.45× c-mount adaptor (Nikon, MQD42040). A nanometer piezo translation stage (PI, P-517.2CL) driven by a digital piezo controller (PI, E-710.4CL) was used, providing the option to generate a look-up table (LUT) to determine the z position of the microspheres.

2.4. Computer

We use a computer with two Xeon E5 2643v2 processors, both containing 6 cores for parallel processing. This computer was used to run a LabVIEW program dedicated for controlling the experimental sequence [10] and to run a MATLAB script to determine the acoustic properties of different dimensions of the system.

2.5. Microsphere tracking

Acquired images were processed in real time to extract the microsphere positions in three dimensions. To determine the x- and y-position, we applied a quadrant-interpolation algorithm [11], whereas for the z position, a look-up table (LUT) was used, which contains a library of radial profiles previously acquired as a function of microsphere z position [12]. The precision of x- and y-position determination was about 1.3 nm, and for z-position determination, it was about 3.8 nm, at an acquisition rate of 60 Hz (Supplementary Fig. 2). Tracking software is freely available (http://figshare.com/articles/AFS_software/1195874).

2.6. DNA Tethering

Both torsional unconstrained pKYBI (8.4 kbp) [13] and lambda (45.5 kbp) DNA tethers were used [10]. Experiments on DNA tethers were all conducted in PBS (138 mM NaCl, 2.7 mM KCl and 10 mM phosphate (pH 7.4); Sigma). Before experiments are conducted, flow cells were treated with polystyrene (3% w/v, Sigma-Aldrich, 331651) in toluene solution to make the surface hydrophobic. This solution was rinsed out with PBS.

For attachment of the pKYBI DNA, the flow cell was incubated with the anti-Dig antibody-containing solution (20 µg/ml, Roche, Cat. No. 11 333 089 001) in PBS for 20 min. A two-step passivation was used, incubating Bovine Serum Albumin (BSA) (0.2% w/v, Sigma-Aldrich, A7906) and then pluronic (0.5% w/v, BASF, pluronic® F 108NF Prill) both 30 min in PBS, reducing nonspecific sticking of the DNA and microspheres. Thereafter, buffer containing the DNA was incubated for 20 min. In the last step, 4.5 µm streptavin coated polystyrene microspheres (0.15% w/v, SVP-40-5, Spherotech, Inc) were flown into the chamber to let them incubate for 20 min.

For attachment of Lambda DNA, biotin-modified casein was produced by reacting casein solution (2% w/v, Sigma-Aldrich, C8654) with an equimolar amount of EZ-Link™ Sulfo-NHS-LC-LC-Biotin (Thermoscientific, 21338) in a borate buffer (pH 8.3) for several hours. The reacted solution was stored at -20C, and could after thawing be used for surface coating. A mixture of biotin-modified casein (0.02% w/v) and casein (1% w/v) in PBS was incubated for 20 min., then streptavin (0.0167 µg/ml, Thermo Fisher, 43-4301) in PBS was incubated for 20 min. Thereafter, the DNA was incubated for 20 min. In the last step, the 4.5 µm streptavidin coated polystyrene microspheres were incubated for 20 min.

3. One-dimensional acoustical model

The analysis of the forces on suspended particles resulting from acoustic pressure dates back to the 1930′s [14] and 1950′s [15].