Testing syringe-pumps by the NanoTracker™ optical tweezers system

Introduction

A number of optical tweezers experiments require laminar flow of liquids in a microfluidic chamber for numerous applications ranging from basic exchange of samples and buffer solutions to advanced experimental configurations and optical trap calibration techniques. There are several types of instrument which can create fluid flows in the sample chamber in very well controlled and adjustable ways such as the precise setting of the flow rates. For optical tweezers experiments, utilization of one or several syringe-pumps is often the most suitable and inexpensive solution.

In designing an optical tweezers experiment where flow is a prerequisite, researchers should ensure that the syringe pumps will make a minimal influence on measurements. A syringe-pump is a mechanical device which usually consists of a stepper-motor, a steering rotor with a thread and a syringe connected to the microfluidic chamber by means of tubing. By steering a threaded rotor, the rotation of the stepper motor is converted into translational movement in order to push or pull the plunger of the syringe. Taking into the account the syringe diameter and varying the speed of the stepper motor rotation, one can adjust with high precision the flow rate of the liquids in the sample chamber. Typical flow rates in biophysical and biochemical applications of the optical tweezers range from 100 µl/min down to a few µl/min. In order to establish such small flow rates even for small-volume syringes the pump should move the plunger extremely slowly which can be a challenging task in itself.

In this report we have used JPK's NanoTracker™ 2 optical tweezers to directly test several models of single- and dual-syringe pumps from World Precision Instruments, (Sarasota, Florida). These included AL1000, AL4000, AL4002.X and SP260p, all of which can be used with NanoTracker™ accessories, such as LaminarFlowCell (LFC™) and PetriDishHeater™. We give also several suggestions, which should be taken into account during preparation of optical tweezers experiments involving syringe-pumps.

Experiment description

We have probed the characteristics of flows produced by syringe-pumps with help of JPK's NanoTracker™ 2 (Figure 1), an off-the-shelf optical tweezers platform designed for high-resolution quantitative force measurements, tracking and nanomanipulation. The NanoTracker™ is equipped with two optical traps, which can both be independently steered and used for tracking or force measurements. Force measurements are performed using back-focal-plane interferometry on InGaAs quadrant photodiodes, which can be calibrated online using the power spectrum analysis feature of the software. The NanoTracker™ software allows full control of all instrument hardware, such as trap/sample positioning, trap stiffness adjustments and also the microfluidic flow.

***Figure 1**. Dual-trap optical tweezers NanoTracker™ 2 combined with Zeiss' Axio Observer inverted-microscope.*

The LaminarFlowCell (LFC™) developed for integrated use in the NanoTracker™ system was utilized as a microfluidic chamber, in which syringe-pumps produced flow rates (see Figure 2). It consists of up to five independent laminar flow channels. These channels can be flexibly laid out and merged: users can individually design their own channel patterns for the LFC™, for example by using polymeric spacers such as Parafilm® or PDMS. The typical thickness of the flow cell (<200 µm) ensures that fluid flow, induced by the software-controlled syringe pumps, is entirely laminar. This means the different input channels will not mix when liquid flows and the boundary between channels remains very narrow (down to a few µm). In conjunction with the use of optical traps, the manipulated particles can be swiftly brought from one channel into the next, simply by moving the microscope stage.

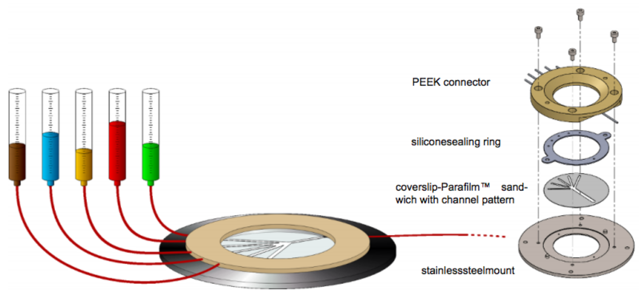

Figure 2. *The schematic illustrates the assembly of the LaminarFlowCell and the geometry in which up to five laminar flows are generated from various syringes.*

In order to test the performance of a syringe-pump in terms of its influence on an optical tweezers experiment, we have used a trapped microparticle as a direct probe of the liquid flow. To do so, a polystyrene bead with diameter of 1.53 µm was trapped and force calibrated 30 µm above the glass coverslip at the bottom of the LFC™. The fluctuations of the bead relative to the trap center were monitored as force signal along the direction of the liquid flow at 2 KHz sampling rate. A range of flow rates from 2 to 30 µl/min was applied for each of the pumps under test and the force response caused by the viscous drag was acquired as 5-10 min time series. Force measurements of the trapped bead fluctuations in the absence of the flow were used as a reference test, where the influence of the syringe-pump is completely excluded but while measurement drifts and system noises were still captured. According to Stokes' law viscous drag forces, which the spherical bead feels under the flow of an aqueous solution, are proportional to the velocity of the fluid movement. Therefore, the measurement of the force applied to the bead by the constant flow in the microfluidics chamber will directly characterize the variations of the liquid velocities, and, in turn, be used to test the performance of a syringe-pump.

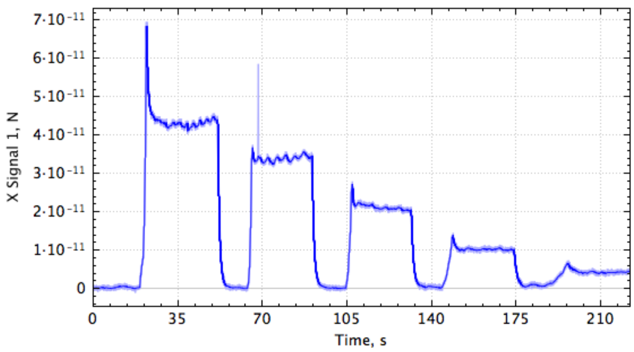

Figure 3. *Measurement of the viscous drag force occurring between liquid flow and optically trapped 1.53 µm polystyrene bead. The flow was established with help of the SP260p syringe-pump. This was repeatedly switched on/off, while the flow rate was varied as follows: 20 µl/min, 15 µl/min, 10 µl/min, 5 µl/min and 2 µl/min.*

Syringe-pump tests

We have probed and estimated the influence of four of WPI's syringe-pumps on the optical tweezers measurements under flow. Namely, these are dual-syringe pumps AL4000, AL4002.X, SP260p and a single-syringe-pump AL1000. Table 1 gives the summary of their specifications as provided by the manufacturer.

Figure 1 shows a time trace of the force, which was exerted on a spherical polystyrene bead due to the microfluidic flow of the water in the LFC™-chamber. The bead was trapped and held in the flow with help of the NanoTracker™ tweezers while the flow rate was changed step-wise from 20 µl/min to 2 µl/min. As one can see, the high force sensitivity of the QPD-based detection system in the NanoTracker™ helps reveal a very detailed picture of the liquid flow established by the syringe-pump. For example, the switching on of the liquid flow is characterized by a short overshoot of the measured force, after which it stabilizes at certain force plateau. This force jump (or flow jump) can be caused by the pressure wave which propagates in the syringes, connecting tubing and microfluidic chamber after a rapid start of the syringe-pump. On the same plot one can see the character of the flow attenuation right after the pump was switched off. Furthermore, the force vs. time trace in Figure 1 demonstrates that the liquid velocity in the chamber can vary slightly, although the flow rate (which is the "pushing" rate of the syringe-pump) was set to a constant value. Such small changes in the liquid velocity can be noticed as variations of the force felt by the trapped bead.

Model	Speed, (min./max.)	Min. flow rate, (1 ml syringe)	Max. flow rate, (60 ml syringe)
AL1000	0.7 µm/min – 5.1 cm/min	0.73 µl/h	28.3 ml/min
AL4000	0.7 µm/min – 5.1 cm/min	0.73 µl/h	28.3 ml/min
AL4002.X	0.014 µm/min – 0.2 cm/min	0.0015 µl/h	1.25 ml/min
SP260p	0.1 µm/min – 1.6 cm/min	0.1 µl/h	8.6 ml/min

Table 1. *Selected specifications of the WPI syringe-pumps.*

In order to estimate and compare the "quality" of the liquid flows that are produced by various syringe-pumps, one can measure the force variations felt by the trapped bead during time periods of several minutes under the same conditions, such as flow rate, separation from the microfluidic chamber walls, bead size etc. Figure 2 shows selected typical force profiles of four WPI's syringe-pumps used to establish the flow rate of 15-20 µl/min in the NanoTracker™'s LaminarFlowCell. The red trace in each plot corresponds to the force exerted on the bead by the flow, while the blue trace is the reference measurement made shortly before with the same bead without any flow. It represents the force signal with no influence from the working syringe-pump and at the same time acquires possible system drift and other sources of noise. It is easy to notice, that force traces for each pump are characterized by certain specific artifacts, which are in turn caused by the variations in the velocity of the flow produced by the pump. Such tiny variations in the flow rates are caused mainly by small imperfections of the pump mechanics although one should not exclude possible influence of the proper binding and filling of the connecting tubing as well as the microfluidic chamber itself.

Due to the high temporal resolution and force sensitivity of the NanoTracker™ 2 system, these experiments demonstrated how to quantitatively compare different syringe-pumps. In turn, this allows the experimenter to directly define the ranges of the pump applications for the desired experimental conditions (e.g. trap stiffness, beads size, flow rates, fluid viscosities etc.). For example, the flow deviations in the micro-fluidic chamber and, subsequently, the noise added to the force measurement for all the syringe-pumps tested were above several pN. This is useful information when experiments should be performed under flow and the expected range of forces is below or close to this value.

Further, one can conclude that for the tested flow rates of 15-20 µl/min the AL4002.X introduces more noise to the force measurement in comparison to other models. This is in agreement with the pump specifications. According to the manufacturer, this pump works at tested flow rates close to the limits of the piston translocation velocities. In turn, these measurements also revealed that the force "finger-print" of every pump model is quite unique and should be taken into account during the experimental design phase. For instance, in the force-trace of the SP260p, one can easily notice the parasitic noise modulated at several frequencies, although the pump outperforms other models in the test in terms of the absolute values of the added noise.

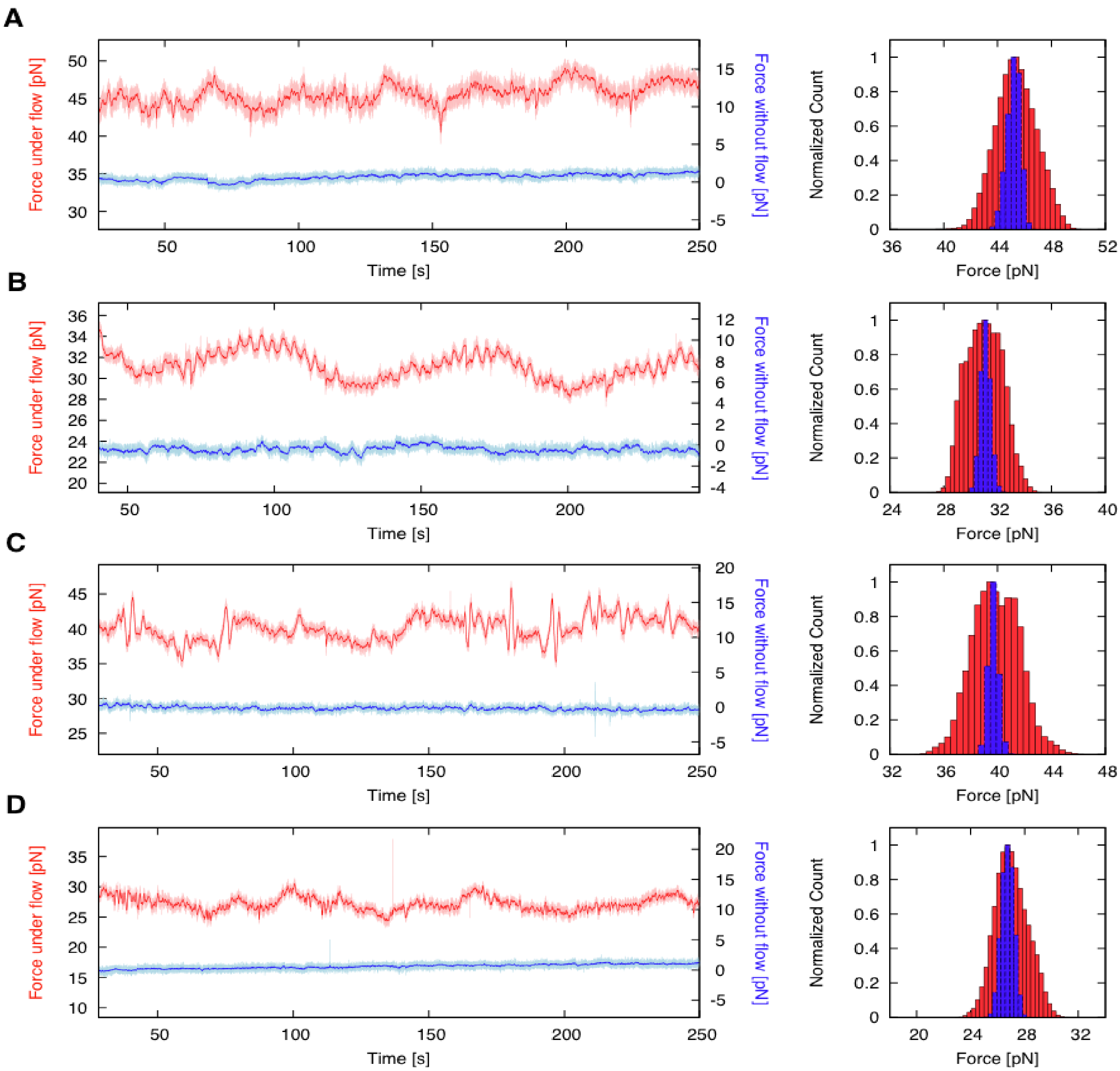

Figure 2. *Force measurements performed on 1.53 µm beads under the flow of four different WPI syringe-pumps: dual-syringe models AL4000 (data panel **A**), SP260p (panel **B**), AL4002.X (panel **C**) and a single-syringe-pump AL1000 (panel **D**). The blue trace in each data set corresponds to the force fluctuations of the bead when flow is switched off, while the red trace is a force exerted on the bead by the flow. The light tones of the color depict the original force signal sampled at 2 kHz, while the dark tones are the rolling average of the same signal.*