Multichannel LaminarFlowCell for optical-tweezers applications

Over the past decade, experiments using optical tweezers have aided in understanding a range of cellular and sub-cellular biological processes, by providing a means to control and measure minute forces on a microscopic scale. Such micromanipulation experiments often involve the sequential addition of multiple different reagents to the solution, in order to controllably set up and trigger biochemical reactions. To this end, microfluidic flow is often employed and integrated in optical-tweezers experiments. In order to offer a complete solution to its customers, JPK Instruments has developed a flow cell dedicated for these demanding applications that is fully integrated in the hardware and software of the NanoTracker™ optical tweezers platform. This note describes the design and use of this LaminarFlowCell (LFC).

Microfluidics LaminarFlowCell

JPK's NanoTracker™ (Fig. 1) is a sensitive and stable force-sensing optical tweezers system. It has many applications in biophysics and biochemistry where precise control over the well-timed addition of reagents is often crucial. In addition, the application of fluid flow can be extremely helpful in applying forces by means of viscous drag, e.g. for extending individual polymeric molecules such as proteins or DNA. The LFC developed by JPK Instruments for integrated usage in the NanoTracker™ system (Fig. 2) 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® (see Fig. 2) or PDMS.

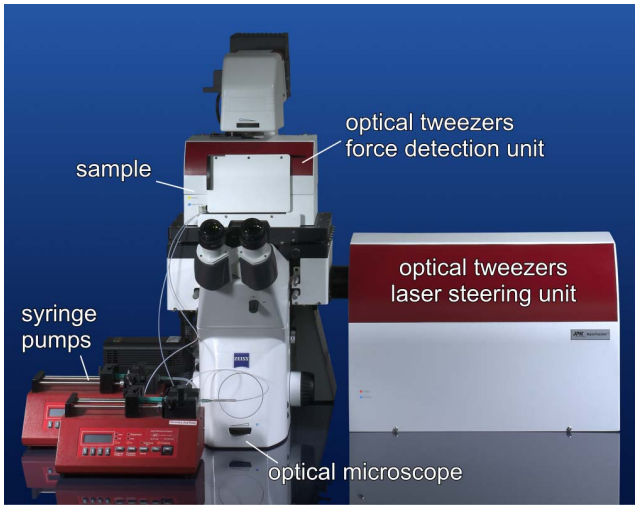

Fig. 1 JPK NanoTracker™; set up here for use with the multichannel laminar flow cell controlled through syringe pumps.

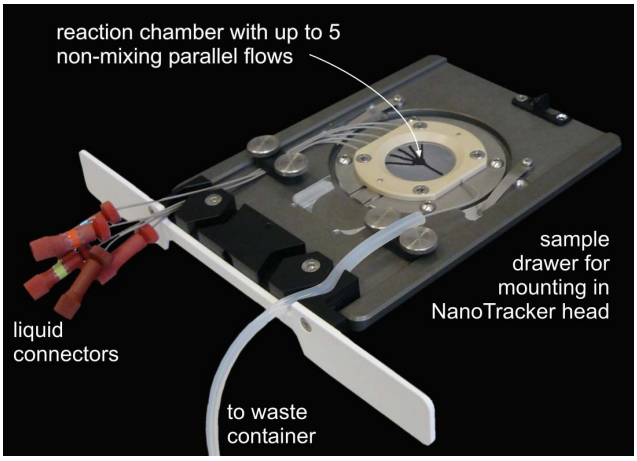

Fig. 2 JPK's Multichannel LaminarFlowCell, mounted in the sample holder of the NanoTracker™, showing the entire sample holder including tubing guides.

The typical thickness of the flow cell (<200 µm) ensures that fluid flow, induced by software-controlled syringe pumps, is entirely laminar. Therefore, 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. This can be clearly seen in the movie available from the JPK website at http://www.jpk.com/newsletter/3-2009/nt-flow-cell.htm. An explanatory overview schematic of the LFC used can be seen in Fig. 3; Fig. 4 shows a microscope image demonstrating the spatial separation of the five channels.

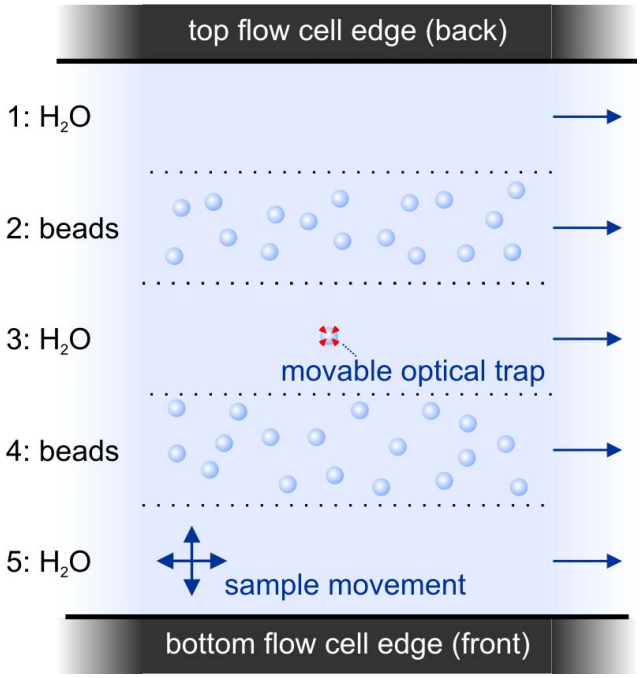

Fig. 3 Channel configuration of 5-channel flow cell.

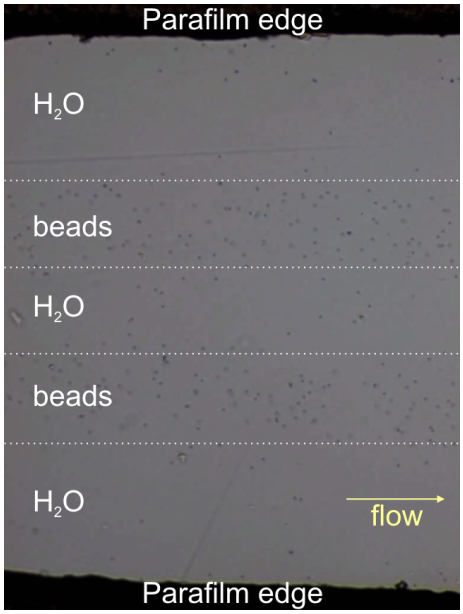

Fig. 4 Overview microscope image showing the 5 channels indicated in Fig. 3. Image recorded using a 4x microscope objective. The corresponding movie can be found on the JPK website at http://www.jpk.com/newsletter/3-2009/nt-flow-cell.htm. In the image, 2-µm beads flow from left to right in two channels, both separated on either side by a channel containing only water. The total width of the five channels is around 2 mm.

Applications

Multichannel microfluidic flow cells have found many applications in biophysics and biochemistry research. Following the first application in conjunction with optical tweezers [1], several reports have appeared that rely on such an integrated approach [2–5]. An in-depth description of the technology was published by Brewer and Bianco [6].

Most applications exploit the availability of multiple channels to rapidly yet reproducibly control the assembly of the required experimental conditions. Galletto et al. caught DNA molecules using optically trapped beads, which they subsequently loaded with fluorescent dyes and then unloaded for further experimentation [2]. Similarly, Noom et al. captured sets of DNA molecules and tested them for mechanical integrity before exposing them to so-called DNA-binding proteins [3]. Van Mameren et al. combined such measurements with sensitive fluorescence measurements, in which the multiple flow channels were used to trigger biochemical reactions by rapidly changing buffer [4] or by controllably loading different fluorescent markers onto the DNA [5].

In all these cases, the application of buffer flow was additionally required for flow-stretching DNA molecules, either to observe them directly using fluorescence, or to facilitate suspending them between two optically trapped particles.

The NanoTracker™ is the first commercially available optical tweezers platform that offers a complete solution for quantitative measurements integrating versatile manipulation, fluorescence visualization, and the sample control through microfluidics described here. JPK's vision has been to finally have optical-tweezers technology become available for scientists that prefer to spend their time on experimentation rather than instrumentation.

Feature summary

In summary, the microfluidics LFC developed for JPK's NanoTracker™ optical tweezers platform features the following:

- Multichannel, flexible design with up to 6 inputs and/or outputs;
- Laminar (non-mixing) flow, keeping channels separated;

- Software-controlled syringe pumps for automated fluid flow and exchange;
- Applications include:
  - Enhanced experimental control;
  - Single-molecule flow stretching;
  - Drag force measurements;
  - Biochemical triggering using sub-second buffer exchange.

Literature

[1] L.R. Brewer, M. Corzett, R. and Balhorn. Protamine-induced condensation and decondensation of the same DNA molecule. Science 286, 120–123 (1999).

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[5] J. van Mameren, P. Gross, G. Farge, P. Hooijman, M. Modesti, M. Falkenberg, G.J.L. Wuite, and E.J.G. Peterman. Unraveling the structure of DNA during overstretching using multicolor, single-molecule fluorescence imaging. Proceedings of the National Academy of Sciences of the USA (2009), in print.

[6] L.R. Brewer, and P.R. Bianco. Laminar flow cells for single-molecule studies of DNA-protein interactions. Nature Methods, 5:517-525 (2008).