LaminarFlowCell (LFC™) heater for optical tweezers applications

Introduction
Optical traps (optical tweezers) allow the contact-free three-dimensional manipulation of nano- to microscale objects and precise force measurements in the sub-piconewton range. Thus, in recent years they have gathered much interest in the field of single-molecule force spectroscopy as well as biophysical applications investigating the mechanics of individual DNA molecules, protein complexes or whole cells. One of the major benefits is the ability to work with suspended particles or other objects like cells or bio-molecules in buffer solution. This eliminates surface effects and provides full flexibility in all spatial dimensions. For experiments involving temperature-sensitive components and expensive substances like purified peptides or proteins, temperature-controlled microfluidic setups are the system of choice. With the re-designed laminar flow cell (LFC™) and the heating option, JPK has developed a system combining stable and precise temperature control with minimal sample volumes for the versatile NanoTracker™ optical tweezers setup.

Superior environmental control for stable and reproducible experimental conditions
Complex experimental setups, for example the investigation of DNA-protein interactions or changes in protein folding dynamics under varying environmental conditions require quick changes of solvents and buffering media. The LFC™ offers the possibility to generate parallel laminar flow of up to five different solutions with minimal mixing and long-term stability. This is achieved by combining multiple separate input channels into one larger measurement channel where due to laminar conditions the fluids run in parallel without turbulent mixing (see figure 2).

Figure 1 LaminarFlowCell (LFC™) with heater and feedback controller. The temperature feedback relies on a platinum resistance thermometer and holds the set temperature stable with an accuracy of 0.1°C. The setpoint can be automatically adjusted via the NanoTracker™ control software and temperature ramps can be easily integrated in an automated experimental workflow.

Figure 2 Top view schematic of the measurement channel combining parallel laminar streams of up to five different media containing e.g. microparticles (beads) and different aqueous solutions.

Within less than a second, biomolecules attached to functionalized particles or whole cells can be transported from one medium to the next by either moving the trap position or moving the microfluidic chamber using a motorized stage or the optional three-axis piezo-driven sample scanner. In the new environment force...
measurements can be repeated in order to document the influence of different salt concentrations, pH or functional molecules on single biomolecule or whole cell mechanics. The LFC™ heater offers additional environmental control in the range from room temperature up to 42°C with a sensor controlled feedback stabilizing the temperature with 0.1°C precision. Enclosed in the NanoTracker™ head unit, the system design reduces thermal drift to a minimum and ensures a stable heat distribution within the measurement channel. The flow velocity within the channels can be precisely controlled via syringe pumps that are accessible through the NanoTracker™ 2 control software. In combination with the ability to run pre-set measurement programs this significantly improves the experimental workflow and ensures reproducible conditions.

**Improved handling and durable materials**
The use of temperature stable and solvent-resistant materials like high grade stainless steel and PEEK (polyether ether ketone) for the heater housing and the HPLC-grade in-/outlet tubing ensures long term integrity of the setup under experimental conditions and makes it suitable for standard sterilization procedures. These include the use of ethanol or isopropanol (whole device) and steam sterilization (base plate only). The microfluidic channels are precision cut in Parafilm® and can be easily replaced or cleaned by rinsing with ethanol and purified water. This carefully chosen material mix also minimizes the emission of toxic substances that might contaminate delicate biological samples and thus affect measurements.

The improved design of the LFC™ components facilitates the quick assembly of leakage-free microfluidic chambers using standard tools. The new heatable LFC™ comes with a water jet cut silicone sealing that ensures tight connections at the chamber in- and outlets. Pre-cut Parafilm® spacers with the standard channel design featuring five incoming and one outgoing (measurement) channel are provided with each system reducing the set-up time to few minutes. Chambers with custom channel designs can be provided by JPK upon request. The flexible channel layout combines several in- and outlet channels and can be adapted to fit any experimental demands.

**Features**
- Multichannel design with up to six in- and/or outlets
- Channel geometry ensures minimized mixing
- Software-controlled syringe pumps for automated flow and fluid exchange
- Optimized components for fast and reliable assembly
- Precision feedback-controlled heater from room temperature to 42°C with 0.1°C stability
- Ethanol-resistant materials for easy cleaning
- Pre-cut Parafilm® channels with variable layout
- Supported applications
  - single-molecule flow stretching
  - temperature dependent single-molecule force spectroscopy
  - live cell applications
  - biochemical triggering based on sub-second buffer exchange

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