Calibrated On-chip Dilution Module for the Multifunctional Pipette

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Abstract:

We have successfully implemented an improved PWFM diluter into the microfluidic pipette platform, highlighting the multifunctionality aspect as a core concept. The main technical improvements are much faster on-off switching of output of the diluter with a time constant of much less than 200 ms, and the ability to dilute two compounds simultaneously. The dilution process is computer-controlled, featuring automated calibration of the diluting stages, and arbitrary concentration sequences at the output of the microfluidic device.

Introduction

Microfluidic device technology has developed into a flexible and versatile laboratory toolbox with high utility in various life science applications. This development is pulled by the increasing demand from biologists and medical researchers, and much accelerated by the push resulting from the rapid innovation in device fabrication and analytical methodology[1]. A particularly challenging research field, where microfluidic devices have contributed to impressive advances, is the study of cellular heterogeneity within animal cell populations[2] [3]. Several research groups have recently reported instances from a new class of microfluidic devices, which can create a hydrodynamically confined flow (HCF) around a microscopic object, such as a membrane patch, or a single cell[4-6]. In contrast to the vast majority of microfluidic devices, where sample processing and detection of analytes occur within closed microchannels, these devices deliver a small liquid volume into an open bath, while simultaneously rerouting the liquid back into the chip. The HCF generated volume, which is fluidically connected to the device tip, can be positioned to stimulate and analyse a single cell, without contaminating the surrounding microenvironment. We have contributed with a refined concept of a microfluidic pipetting device, which utilizes the HCF principle[7]. Our configuration enables recirculation while allowing for angle adjustment and repositioning of the device. It is closely related to the utility of micropipettes[8], which are commonly used in microscopy experiments. This positioning feature, in combination with the ability to easily integrate microfluidic functionality into the device, strongly influenced the decision to coin our concept a ‘multifunctional pipette’.

The on-chip flow circuitry is of special importance when pharmacological aspects, such as cell-to cell variation of drug sensitivity[9], are in the center of the single-cell experiment. In this context, dose-response investigations require the generation of concentration gradients,

which represents a particular strength of microfluidic devices. Accurate gradient generation allows quantitative measurement of cellular response, which is invaluable to address fundamental biological questions, since chemical gradients are a key factor in many biological processes, such as cancer metastasis, immune response, and others[10].

We previously designed and characterized a fast and precise multistage dilution module for microfluidic devices, based on the pulse width flow modulation (PWFM) principle[11]. We reported a device capable of diluting up to 100 fold, and experimentally determined a set of rules that can be conveniently utilized to design multistage diluters. The device was initially utilized in soft matter research to determine the effective calcium concentration required for formation of flat giant unilamellar vesicles from multilamellar phospholipid reservoirs on silica surfaces.

Here we present a functionally enhanced microfluidic diluter module, which is intended to be integrated into the multifunctional pipette concept (**Figure 1A**). The new chip design features two 10:1 dilution stages for gradient generation, and an additional stage close to the channel outlet, which enables rapid switching between the diluted active compound stream and solvent (**Figure 1B**). The solvent of choice is typically the aqueous buffer which constitutes the external bath solution. The diluter module is mounted on a holding interface, which is similar in design and function to the manifold/holding arm of the multifunctional pipette[7]. The diluter is interfaced to a computer controlled pressure generator, which supplies moderate pressure for the device integrated solution wells, as well as for the high pressure pulses which actuate the pneumatic valves for flow control. By means of micromanipulation equipment, the entire assembly can be positioned next to the object of interest, typically single adherent cells in a microscopy environment. In the focus of our current work were the design, fabrication, implementation and calibration of this multi-stage PWFM module.

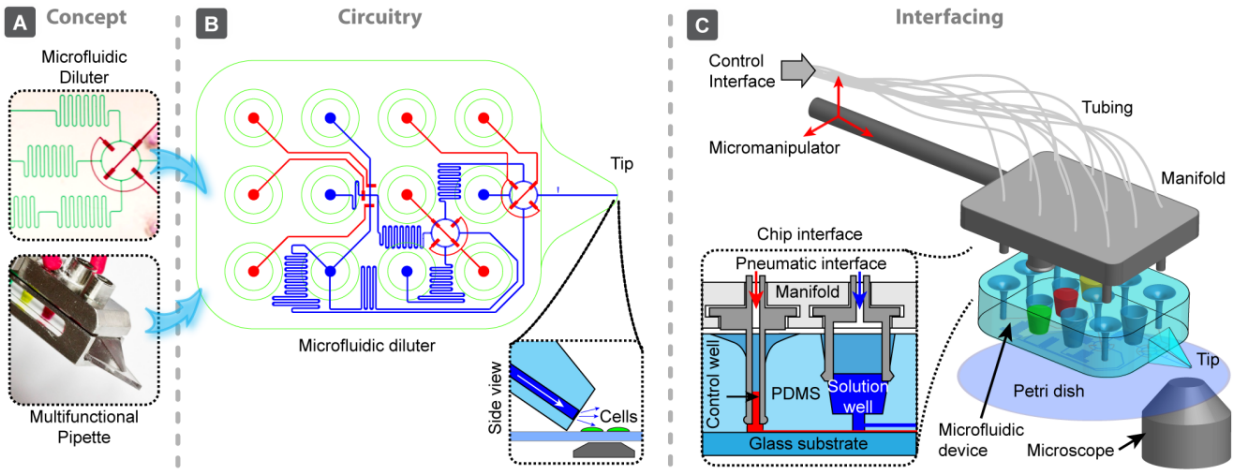

Figure 1. *Technological concept, microfluidic circuitry and interfacing strategy for the on-pipette dilution module.* **A.** *The concept as a combination of the multifunctional pipette design with a PWFM diluter module.* **B.** *The PWFM diluter schematics, with flow channels (blue) and control channels (red). The device consists of two sequential dilution stages, located in the center of the chip, and a third switching stage close to the tip. The first diluter stage is in T-configuration, which allows diluting two liquids simultaneously, while the second stage is in O-configuration, diluting a single input stream. The solvent used for dilution and switching in all three stages is supplied from the same reservoir. The inset shows schematically how the tapered tip is positioned next to the target objects in a typical microscopy setup.* ***C.*** *The interfacing*

strategy with the PDMS chip device (light blue), the pressure manifold/holding arm and the positioning under a microscope (microscope objective not to scale). The inset shows a magnification of the chip/manifold interface, featuring push-in connections which are narrow and deep for the high pressure switching (control wells) and wider, shallower low pressure connections for the solution wells.

Materials and Methods:

Device design: The device consists of two dilution stages, where the first stage is a T-shaped mixer and the second one an O-shaped mixer. Both use pulse width flow modulation (PWFM)[11] for mixing the solvent with the concentrated solution of the active compound. A third O-shaped stage was placed close to the output. The short distance to the tip enables changing the concentration at the output in less than 0.5 s. The over-all fluid-channel length is 101 mm, the channel height and width are 22 μm and 100 μm, respectively. As the first stage has three inflows (T-configuration), thus it is possible to dilute two different liquids A and B simultaneously. Note that the ratio between A and B cannot be smaller than 0.1 in this 10:1 diluter stage.

Device fabrication: The device fabrication was carried out as described by Ainla *et al.*[11]. In brief, mold masters for the control and fluidic channel layers were fabricated by means of photolithography. These molds were subsequently utilized for a soft lithography step, using a two component poly-dimethyl siloxane (PDMS) elastomer kit (Sylgard 184). The only major difference to the previously described process was the use of a specially constructed casting chamber, which defined the solution and control wells in the desired positions with respect to the channel structure. The connections between the channels and the wells were established by punching through a thin residual layer at the bottom of the wells. The bottom of the wells was finally closed by bonding of the PDMS body to a 1mm glass substrate (*cf.* Figure 1C, inset).

Device calibration: The calibration experiments were performed using a Leica Model DM IRB inverted microscope. A *Chameleon CMLN-13S2C* camera (Point Grey Research Inc., Richmond, Canada), which features a 12 bit AD-converter and a resolution of 1296x964 pixels, was used to image the fluorescence intensities. Details on the valve actuation, performance measures and pressure control hardware can be found in[11]. Source code for the Labview® calibration routine is available upon request from the corresponding author.

Results

Device samples were fabricated according to the modified procedure outlined above, and interfaced to the home-built pressure device with Labview interface. **Figure 2A** shows schematically the calibration and deployment concept. In a calibration run using fluorescein solution, the relationship between duty cycle and fluorescence intensity, which is a direct measure of fluorescein concentration, was determined and stored in a calibration file (top panel), which can subsequently be used for dilution of a desired active compound (bottom panel). In the investigated dilution range (10 μM−1 mM in aqueous solution), the fluorescence intensity is linearly dependent on fluorescein concentration. Note that higher concentrations might lead to might yield quenching or reabsorption. The calibration file was generated with in-house developed Labview® software (**Figure 2B**), which also communicated with the pressure controller and camera hardware (**Figure 2A**),

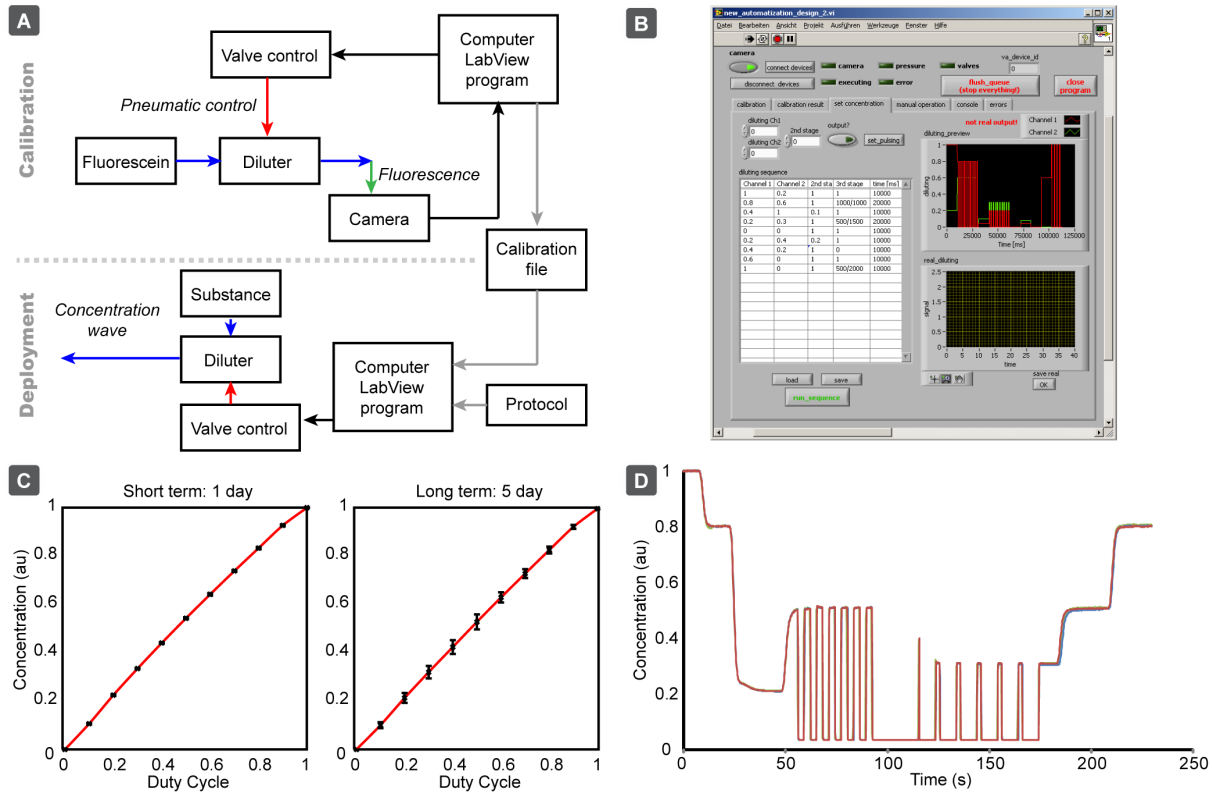

Figure 2. A. *Calibration/Deployment scheme for the microfluidic diluter module. Red: control channel actuation; blue: fluid flow; green: fluorescence signal; black: electronic signals; grey: experimental parameters.* **B.** *Screenshot of the main Labview® control interface.* **C.** *short term and long term calibration curves for fluorescein dilution, showing the relationship between fluorescence intensity (concentration measure) and duty cycle. 15 experiments were performed, the error bars represent 2 standard deviations. Details are discussed in the text.* **D.** *Three nearly identical successive switching sequences (overlaid graphs in red, green and blue). In the first section (t = 0-50s), the dilution is increased stepwise to 20% of the initial concentration, and afterwards increased to 50%. In the second section (t = 50-100 s), fast pulsing, and in the third section (t = 100-175 s), slow pulsing is demonstrated. In the forth section, from t = 175 s onwards, dilution is reduced stepwise until the original concentration is re-established.*

Chip calibration was performed diluting a fluorescein-solution (1.026 mM) in Milli-Q water. In a typical calibration experiment, the period of valve switching was 500 ms, and the duty cycle was modified in 50 ms steps to get 11 calibration points (**Figure 2C**). The pressure in the fluidic channels was set to 0.40 bar. For the calibration of the second stage, the switching time ratio first one was set to 50:450 ms. Two different aspects were investigated: In a first set of experiments, the reproducibility of calibrations was tested by recording 13 calibrations over a time period of 3.5 h (left panel). In a second set, information on long time and production stability was obtained (right panel). 15 calibrations were recorded on 5 different days, employing 2 different devices. The figure shows the calibration curves obtained for the first stage. **Figure 2D** depicts an exemplary deployment sequence of solution delivery (1mM fluorescein stock solution, fluorescence readout), demonstrating both concentration adjustment of the two-stage diluter with a time constant of about ~4s, and fast on-off switching of the diluter output by using

the 3rd (switching) stage. The time constant here is much smaller than the smallest shutter time of our camera (~200 ms). The figure contains three repeated sequences (overlaid graphs), showing acceptable repeatability with only minimal run-to run deviation.

Summary and Conclusion:

We have successfully implemented an improved PWFM diluter into a microfluidic pipette platform. This development highlights the multifunctionality aspect of the core concept which we have repeatedly emphasized[7, 12]. The main technical improvements are much faster on-off switching of output of the diluter with a time constant of much less than 200 ms, and the ability to dilute two compounds simultaneously. This extended functionality reaches its highest utility through the implementation into the pipette design, allowing free positioning towards an object of interest on a surface. The dilution process is computer-controlled, which features automated calibration of the diluting stages, and arbitrary concentration sequences at the output of the microfluidic device. The diluter module is ready to be transferred into the current HCF pipette system, where it shows promise to be able to facilitate single cell studies, enabling precise and reproducible gradient generation in a spatially confined solution environment.

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