FLUIDIC STRUCTURE FOR TEMPERATURE MEASUREMENT UNDER ROTATION IN CENTRIFUGAL MICROFLUIDICS

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ABSTRACT

We present a novel fluidic temperature measurement system (FTMS), which allows measurement under rotation at arbitrary positions. Temperature determination and control is of high importance for many biochemical applications integrated into centrifugal microfluidic (CM) cartridges while the integration of electronic temperature sensors into processing devices remains a challenge. By enclosing a well-defined air volume inside a fluidic chamber, temperature-induced volume expansion is utilized to displace fluorescent liquid into a detection chamber. Results of a proof-of-concept are in good accordance with temperatures of a commercially available thermocycler (Rotor-Gene Q, QIAGEN GmbH, Germany).

KEYWORDS: centrifugal microfluidics, temperature measurement, PCR, melt curve analysis

INTRODUCTION

Centrifugal microfluidics (CM) proves to be an attractive platform for automation of biochemical applications [1], of which many require precise temperature control of reaction volumes. It is especially crucial for nucleic acid testing employing amplification methods e.g. polymerase chain reaction (PCR) [1] and for fluidic unit operations based on temperature effects [2]. Temperature measurement via e.g. infrared (IR) sensors may be suitable for spin-stands [2] but their integration into processing devices may be challenging. Especially temperature determination of minute liquid volumes at arbitrary positions on a disk under rotation renders infeasible due to resolution and sampling rate limitations of common IR sensors. The integration of a fluidic structure into the CM cartridge itself, capable of measuring the temperature at positions, inside geometries and liquid volumes of interest, enables precise measurement of temperature under rotation. We demonstrate a proof-of-concept by measuring temperatures during a melt curve analysis inside the rotary thermocycler Rotor-Gene Q (RGQ, QIAGEN GmbH, Germany).

THEORY

Volume expansion $\Delta V(T)$ of a saturated air volume V_1 enclosed at atmospheric pressure p_{atm} and room temperature T_1 with temperature increase to T is described below,

$$\Delta V(T) = \frac{(p_{atm} - p(T_1)) * V_1 * (T + 273.15)}{(p_{atm} - p(T)) * (T_1 + 273.15)} - V_1 \quad (1)$$

where p(T) represents the vapor pressure of water at temperature T as described below,

$$p(T) = 10^{8.07131 - \frac{1730.63}{233.426 + T}} * 133.322 \quad (2)$$

and is plotted in Fig. 1 (blue line). Volume expansion of 40 µl, 50 µl, 60 µl, and 70 µl (red dotted lines), respectively, is assumed to be similar to the displaced liquid volume at sufficiently low rotational speed (400 rpm) and thus yields the temperature of expected fluorescence signal increase inside the detection chamber of a FTMS with 40 µl, 50 µl, 60 µl, and 70 µl siphon volume (Fig. 2), respectively.

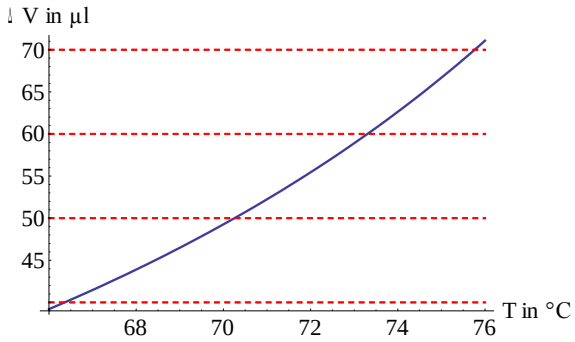

Figure 1: Calculation of temperature (intersection of blue and red curves) at which 40 µl, 50 µl, 60 µl, and 70 µl (red dotted lines), respectively, of volume expansion $\Delta V(T)$ of an initial saturated air volume V_1 = 79.7 µl at room temperature T_1 = 24.4 °C and atmospheric pressure p_{atm} = 100.6 kPa is expected.

EXPERIMENTAL

The fluidic temperature measurement system (FTMS) is micro-thermoformed according to [3] (Fig. 2, left) and consists of an inlet chamber (199.7 µl) filled with 120 µl of deionized (DI) water with 100 µM fluorescein amidite (FAM) (Fig. 2a, right). Subsequently, the RGQ is started at room temperature (24.4 °C) rotating the FTMS at 400 rpm, thereby enclosing a well-defined air volume (79.7 µl) inside the unvented inlet chamber (Fig. 2b, right). During melt curve analysis, the temperature inside the calibrated RGQ is increased by 1 °C/4 s from 50 °C to 80 °C, leading to expansion of the enclosed air volume and displacement of the liquid through different siphon volumes into detection chambers (Fig. 2c d, right). Fluorescence is measured after each temperature increase of 1 °C. Each siphon volume (and fill-in volume) corresponds to a defined measurable temperature. The siphon volume was varied from 40 µl to 70 µl in 10 µl increments to measure a range from 65 °C to 75 °C.

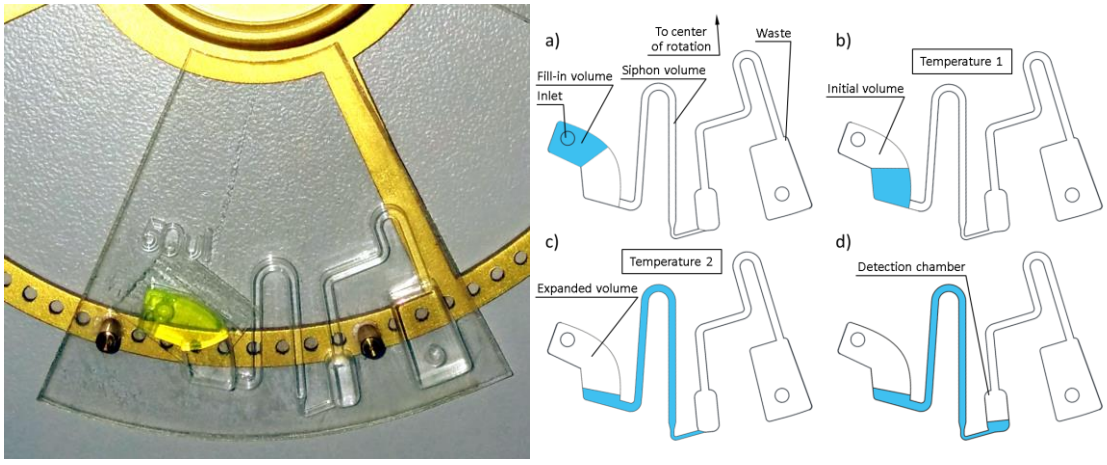

Figure 2: Left: Picture of the fluidic temperature measurement system (FTMS) structure (segment of a disk) inside a corresponding holder (gold-colored) indicating the siphon's volume (here: 50 µl). Right: Process flow. (a) The FTMS comprises an inlet chamber of well-defined volume that is connected to a detection chamber via a siphon channel of well-defined volume. The inlet chamber is unvented; the detection chamber is vented via a waste structure. A well-defined volume of DI water with fluorescein amidite (FAM) is inserted into the inlet and the inlet is sealed gas-tight afterwards. (b) Upon rotation of the Rotor-Gene Q (QIAGEN GmbH, Germany) the filled-in liquid is transferred radially outward thereby enclosing a well-defined air volume V_1 at room temperature 1 T_1 = 24.44 °C. (c) During temperature increase the enclosed air volume expands and displaces the liquid into the detection chamber at a temperature 2, which results in an abrupt signal increase at the fluorescence detector. (d) The fluorescence signal increases and reaches its maximum as the detection chamber continues to fill.

RESULTS AND DISCUSSION

The displayed temperatures of the RGQ at abrupt fluorescence increase, which are assumed to be sufficiently accurate during melt curve analysis, are taken as measured values (Fig. 3). They are in good accordance with the calculations (Fig. 1, Tab. 1).

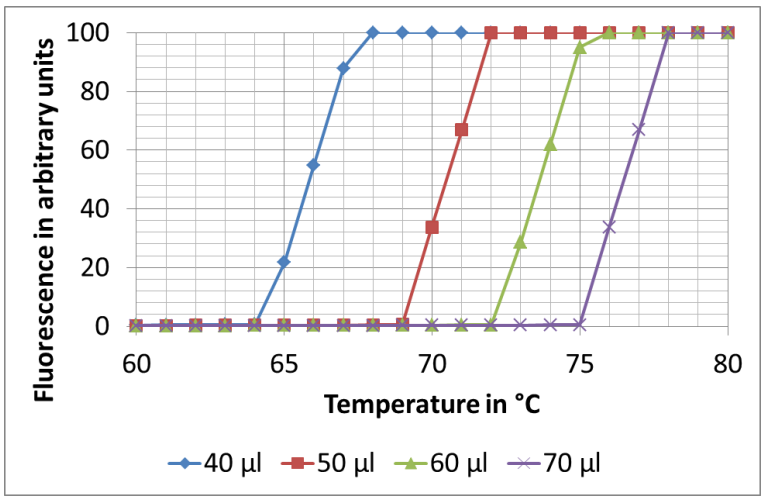

Figure 3: Sudden fluorescence signal increase during melt curve analysis inside the detection chamber of the fluidic temperature measurement system (FTMS) with a 40 µl (blue), 50 µl (red), 60 µl (green), and 70 µl (purple) siphon volume, respectively, at a distinct temperature.

Table 1. Comparison of measured (Fig. 3) and calculated (Fig. 1) temperatures.

Comparison measurement and calculation	**Siphon volume in µl**			
	40	**50**	**60**	**70**
Measured temperature in °C	65	70	73	76
Calculated temperature in °C	66.4	70.3	73.3	75.8

As inaccurate fill-in volumes may directly lead to falsified temperature measurements, a metering step prior to the temperature measurement may be integrated fluidically in the future.

CONCLUSION

The proof-of-concept shows great potential for the FTMS to be directly integrated into CM cartridges for biochemical applications e.g. our *LabDisk* platform. By determination of temperature under rotation at the point of interest, integration and control of thermal protocols for e.g. nucleic acid amplification methods may significantly be simplified. As the FTMS is designed to reversibly fill and empty the detection chamber it is regarded suitable for measuring temperatures during thermocycling for e.g. PCR.

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