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**Garbarino, Francesca; Minero, Gabriel Khose Antonio; Fock, Jeppe; Rizzi, Giovanni; Neumann, F.; Madaboosi, N.; Asalapuram, P.; Nilsson, M.; Hansen, Mikkel Fougt**

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# Automated rolling circle amplification and optomagnetic product detection in an injection molded all-polymer chip – optimization of amplification temperature

F. Garbarino<sup>a</sup>, G.A.S. Minero<sup>a</sup>, J. Fock<sup>a</sup>, G. Rizzi<sup>a</sup>, F. Neumann<sup>b</sup>, N. Madaboosi<sup>b</sup>, P. Asalapuram<sup>b</sup>, M. Nilsson<sup>b</sup>, M.F. Hansen<sup>a</sup>

<sup>a</sup>Department of Micro- and Nanotechnology, Technical University of Denmark, DTU Nanotech, Building 345B, DK-2800 Kgs. Lyngby, Denmark

<sup>b</sup>Science for Life Laboratory, Department of Biochemistry and Biophysics, Stockholm University, Box 1031, Se-171 21 Solna, Sweden

e-mail: mikkel.hansen@nanotech.dtu.dk

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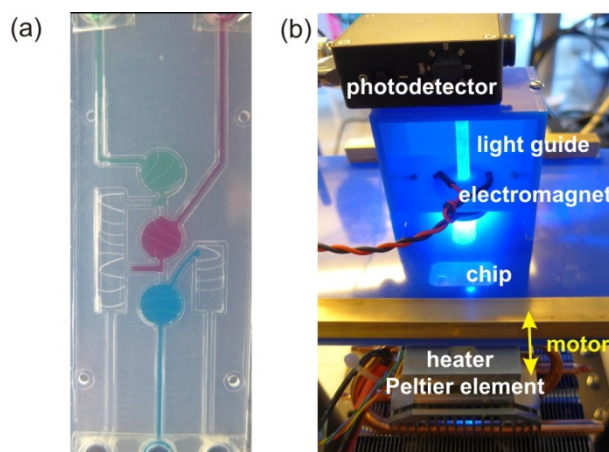
We present an injection molded polypropylene (PP) chip with passive liquid handling designed for the automation of an isothermal rolling circle amplification (RCA) assay. Furthermore, we demonstrate on-chip optomagnetic (OM) readout of the synthesized rolling circle products (RCPs) based on their size. For this, we use a type-B influenza virus synthetic target and optimize the RCA temperature for achieving enhanced RCP size. The work shows the feasibility of integration of the OM readout with a multichamber injection-molded chip with temperature control and presents the results of a pilot study towards a complete on-chip assay.

In RCA, padlock probes hybridize to the matching target and form circles upon ligation. These circles act as the template for linear isothermal amplification using phi29 polymerase. A long RCP, with repeats of the sequence complementary to the circle, is formed [1]. The efficiency of the amplification, in terms of RCP size, is highly influenced by the reaction temperature.

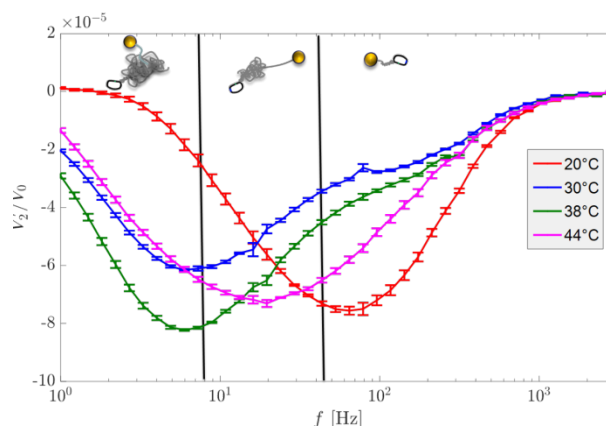
The polymer chip consists of two 1 mm thick injection moulded parts of PP polymer (PP grade RF366MO) that are ultrasonically welded to each other [2]. The chip layout features three circular chambers ( $\varnothing$  5 mm) of height  $H = 200 \mu\text{m}$  with a sequence of phaseguides of height  $h = 60 \mu\text{m}$  to control and enable filling of each chamber with a different liquid (Fig. 1a) from the inlets. Each chamber is connected to a waste chamber, which is then connected to an outlet. The three chambers enable future integration of the complete assay on the chip, but in this work they are filled with the same liquid.

The automation and measurement setup consists of a motorized stage that shifts the chip position between (1) a heater bed regulated by a Peltier element and (2) a position for transmission OM measurements ( $\lambda = 405 \text{ nm}$  LED, photodetector, two electromagnets) (Fig. 1b). RCA mixture contains ligated circles hybridized to the biotinylated target and attached to streptavidin magnetic nanoparticles (MNPs), phi29 and other reagents for amplification. After loading the chip with RCA mixture, the inlets and outlets are sealed using tape. The chip is then placed in position (1) at temperature  $T$  and RCA is performed for 20 min followed by quick cooling to  $25^\circ\text{C}$ . Then, the motor stage shifts the chip to position (2), where the OM spectra are recorded. The entire procedure is automated in LabVIEW, with each cycle (from loading the chip on the setup to the last measurement) lasting around 30 min.

The OM technique relies on measurements of the 2<sup>nd</sup> harmonic modulation of the light transmitted through a suspension of MNPs vs. frequency of an applied magnetic field. In the resulting OM spectrum ( $V_2/V_0$ ) a peak is centered at a frequency, which is inversely proportional to the hydrodynamic volume of the MNPs [3]. In this work, we studied the spectra after amplification for 20 min at  $T$  ranging from 20 to  $44^\circ\text{C}$  to determine the value of  $T$  resulting in the most efficient RCA in terms of RCP size (i.e. the largest RCP corresponding to the lowest value of the peak frequency) (Fig. 2). Excessively low temperature ( $\sim 20^\circ\text{C}$ ) results in low enzyme activity while too high temperature ( $>42^\circ\text{C}$ ) deactivates the enzyme. The results show that the largest product was obtained for  $T = 38^\circ\text{C}$ . Future work aims to integrate on-chip RCA and OM readout with sample preparation (extraction and ligation).



**Figure 1** (a) Photograph of the ultrasonically welded chip with three chambers ( $\varnothing$ 5mm) filled with different dyed liquids. (b) Setup used for heating and OM detection on polypropylene chip.



**Figure 2:** OM spectra of suspensions of MNPs with attached targets/RCPs measured at  $20^\circ\text{C}$  after 20 min of RCA. The figure shows representative results obtained for  $T = 20, 30, 38$  and  $44^\circ\text{C}$ . The frequency of the peak is inversely proportional to the hydrodynamic size of the MNPs.

1. M. Nilsson *et al.*, Science 265, 2085-8 (1994).
2. K. Kistrup *et al.*, Lab Chip 15, 1998-2011 (2015).
3. J. Fock *et al.*, Phys. Chem. Chem. Phys. 19, 8802–8814 (2017).

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