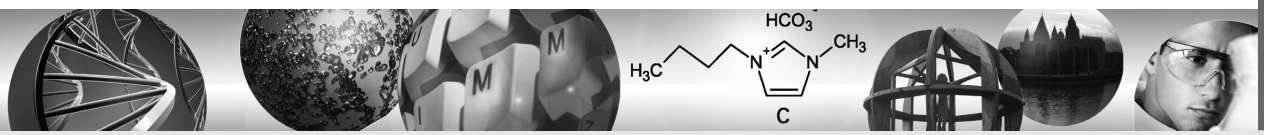

Automatic Detection of Disease Related Molecular Cell Activity

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Introduction

The diagnosis of many common diseases requires sending samples to specialised labs for processing. Besides increasing costs this also takes time, increasing patient anxiety and delaying the start of treatment. It is thus desirable to carry out the diagnosis at the local doctor's office. A reliable way to achieve this is by using bio-marker mRNA detection, since mRNA detection avoids false positive results if marker activity is the desired target and has a high sensitivity. Currently, this technique is among others available for early detection of cancer and a range of respiratory diseases but new biomarkers emerge regularly.

As an exemplary detection system this project aims at detection of infections with a group of viruses causing cervical cancer. Cervical cancer is the second most predominant form of cancer among women in developed countries [1]. Nearly all cases of this cancer are directly linked to previous infection with one or more of cancer-inducing types of the human papilloma virus (HPV) [2]. Detection of persistent HPV infection with oncogene expression would identify women who may need treatment at an early stage.

Against this background, a chip-based automated platform for the extraction of nucleic acids, including HPV mRNA from cervical smears, has been developed (figs.1, 2).

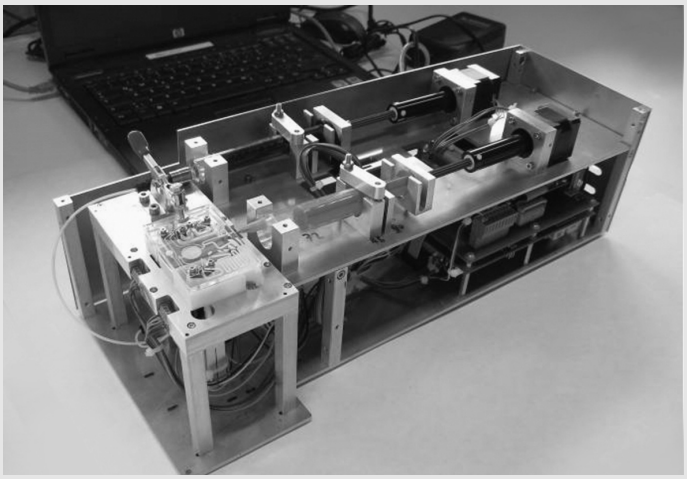


Figure 1: Operating device for the automated extraction of mRNA containing two syringe pumps for fluid actuation, motors for valve operation, a heater and several light barriers for motor control and fluid positioning as well as the electronic control. The device is operated by a customized LabView program.

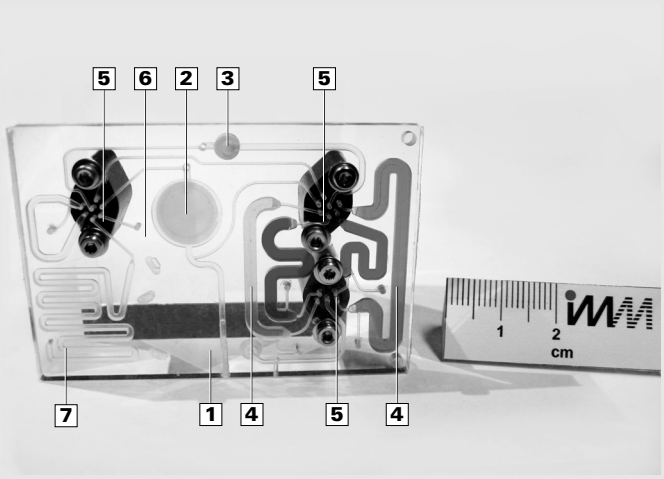
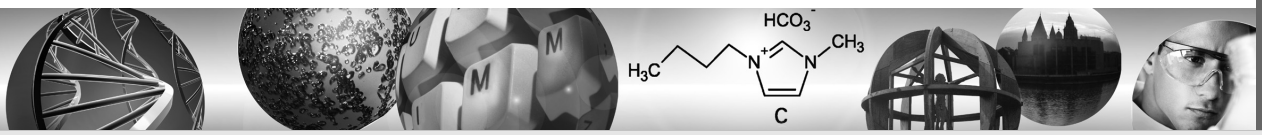


Figure 2: Chip for sample preparation. The basic functional units contain (1) sample inlet, (2) cell filter, (3) SPE chamber, (4) reagent storage section, (5) turning valves, (6) waste outlet, (7) sample outlet.

Competences

- Transfer of macro-scale analytical assay to chip format
- Prototyping of automated device for LOC operation
- Biological validation of extraction procedure

Automatic Detection of Disease Related Molecular Cell Activity



Setup

The device accepts 2-5 ml of a suspension of fixated cervical smear cells in a methanol based solution (PreservCyt™). The sample is injected into a disposable chip (fig. 2) by a syringe and cells are collected on a nylon filter as first step. These cells are then chemically lysed by flushing a high-molar caotropic salt solution through the filter chamber. The lysate passes a solid phase extraction (SPE) chamber containing a stack of silica filter membranes, where the mRNA is retained. Two washing steps remove cell debris and salt. After air-drying of the filter stack, the captured RNA is eluted by rinsing with water. In order to minimize user interference during the sample preparation procedure all liquids necessary for the operation are stored on chip.

The fluid actuation is maintained by two syringe pumps (fig.1). The sample is supplied in a disposable syringe. A second reusable syringe is used for pressure driven actuation of assay reagents as well as for air-drying before elution. The fluid control is performed by three turning valves. In order to obtain an exact timing and positioning of the plug for further processing, a light barrier detects the arrival of the eluate at the sample outlet. A heater elevates the temperature both during cell lysis and air-drying of the SPE chamber. Furthermore, a pressure sensor detects an excessive pressure build up during sample loading so that the capacity of the cell-filter is not exceeded.

At present, the device has been successfully tested on HeLa cell lines which express HPV-18 mRNA. In order to validate the device performance the eluate from the instrument was amplified by Nucleic Acid Sequence Based Amplification (NASBA) using the PreTect® HPV-Proofer kit (NorChip AS, Klokkarstua, Norway). Presently, the device is investigated and optimized for its robustness and extraction efficiency as well as its performance with clinical samples.

Summary

The project MicroActive aims at integrating the sample preparation device presented here with a second automated instrument for on-chip parallel NASBA amplification and detection of several mRNA targets exemplified by different HPV types [3,4]. This combined system may thus serve as a point of care system for the detection of gene expression directly in a physician's office, avoiding the often delayed analysis by a specialized laboratory.

However, the presented sample preparation device is not limited to cervical samples and opens the way for a range of similar sample treatment applications. With small modifications these systems can be adapted to other fields of operation where it is desirable to analyse complex biological samples "in the field" and on a short timescale. This includes for example

- Foodstuff analysis / animal feed control
- Medicine (personalised Medicine, Point-Of-Care)
- Forensics

Project Partners

NORCHIP

Coombe Womens' hospital (Ireland), SINTEF (Norway), IMTEK (Germany), BioFluidix (Germany)

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Institut für Mikrotechnik Mainz GmbH

Contact: info@imm-mainz.de
http://www.imm-mainz.de