A CHEAP 2D FLUORESCENCE DETECTION SYSTEM FOR µM-SIZED BEADS ON-CHIP

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ABSTRACT

In this paper a compact fluorescence detection system for on-chip analysis of µm-sized beads is presented. The system comprises a cheap optical pickup with integrated functionalities that are used for the detection. Furthermore some other optical components are added for the fluorescence detection. Another part of the system is the microfluidic chip which contains liquid channels and optical markers used for the autofocus and channel find algorithms. Within one minute the channel of interest is found and fluorescent beads are detected in the microchannel without the need for dynamic focusing, since a two-dimensional scan across the channel width is performed.

KEYWORDS: Fluorescence, detection, beads, microfluidic chip, optical markers, 2D

INTRODUCTION

Flow cytometry is widely used for the measurement of cell characteristics, requiring a large and expensive system that needs trained personnel. To overcome these disadvantages and to make it suitable for lab-on-chip applications, we developed a compact fluorescence detection system for on-chip analysis, which consists of a low-cost HD-DVD pickup. A DVD pickup was already used for fluorescence analysis in a DNA chip [1], the study of microfluidic properties and microsurfaces [2] and cell detection by measuring the extinction using a mirror [3]. Unlike these studies we used a HD-DVD pickup in combination with a microfluidic chip to measure the fluorescence in a microfluidic channel in two dimensions, resulting in a fluorescence profile across the channel.

DESIGN

The design consists of two parts: the fluorescence detection system and the microfluidic chip (figure 1 and 2). For the fluorescence detection the widely available PHR-803T pickup is used, consisting of several optical components. It has a 405nm ultraviolet laser diode besides the dual emitting laser diode for CD and DVD formats. In table 1 the standards of the formats are shown, indicating that the highest resolution is achieved with the HD-DVD format. Due to this high resolution, the depth of focus is small, meaning that positioning of the microfluidic chip is important. This is achieved by autofocus that is incorporated in the PHR-803T using the astigmatic principle. The photodetector of the pickup cannot be used for the fluorescent measurements and therefore a semiconductor photomultiplier has been used (Hamamatsu S10362-11-100U). A Labview in-

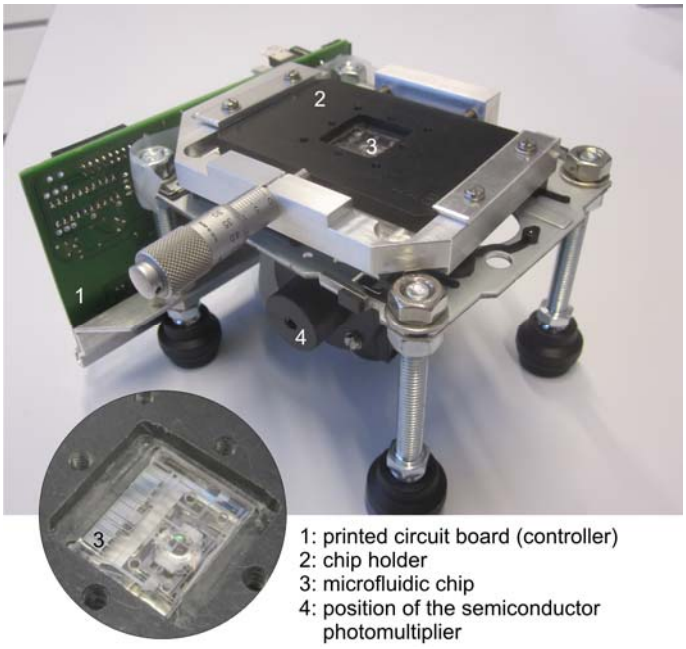

Figure 1: A photograph of the fluorescence detection system with the microfluidic chip. The top of the chipholder (2) is removed to show the chip.

terface and a microcontroller program have been developed to configure the settings, find the fluidic channels and perform the measurements. The glass-glass chip consists of three microchannels that are 18µm and 100µm in depth and width respectively. The chip has optical reflective platinum markers, needed for the autofocus and the implemented channel find algorithm

EXPERIMENTAL

First the autofocus and channel detection of the system was tested. Subsequently 6µm peakflow cytometry reference beads (Invitrogen P12826) were suspended in medium, guided in the microchannel and fluorescently detected. The same experiment was done with 2.5µm fluorescent beads (Invitrogen BLA) and finally with 6µm non-fluorescent beads (Poly BLA).

RESULTS AND DISCUSSION

Within 1 minute the focus of the laser is automatically positioned in the middle of a chosen channel on the chip. Typical measurements of the autofocus and channel detection are shown in figure 3. The channel width measured by scanning in horizontal direction agrees well with the actual dimension on the chip. Only fluorescent beads were optically detected with the system and the detection of 6µm fluorescent beads is shown in figure 4, where a 2-dimensional cross section of the microfluidic channel is shown containing beads. This indicates that fluorescent labeled cells can also be detected. By placing electrodes in the microchannel, electrical impedance measurements can be performed simultaneously, giving additional information about the particle or cell properties.

CONCLUSION

A low-cost HD-DVD pickup can be used for real-time 2-dimensional fluorescence measurements on chip. Further investigation is focused on measurements on cells and incorporating electrical impedance measurements.

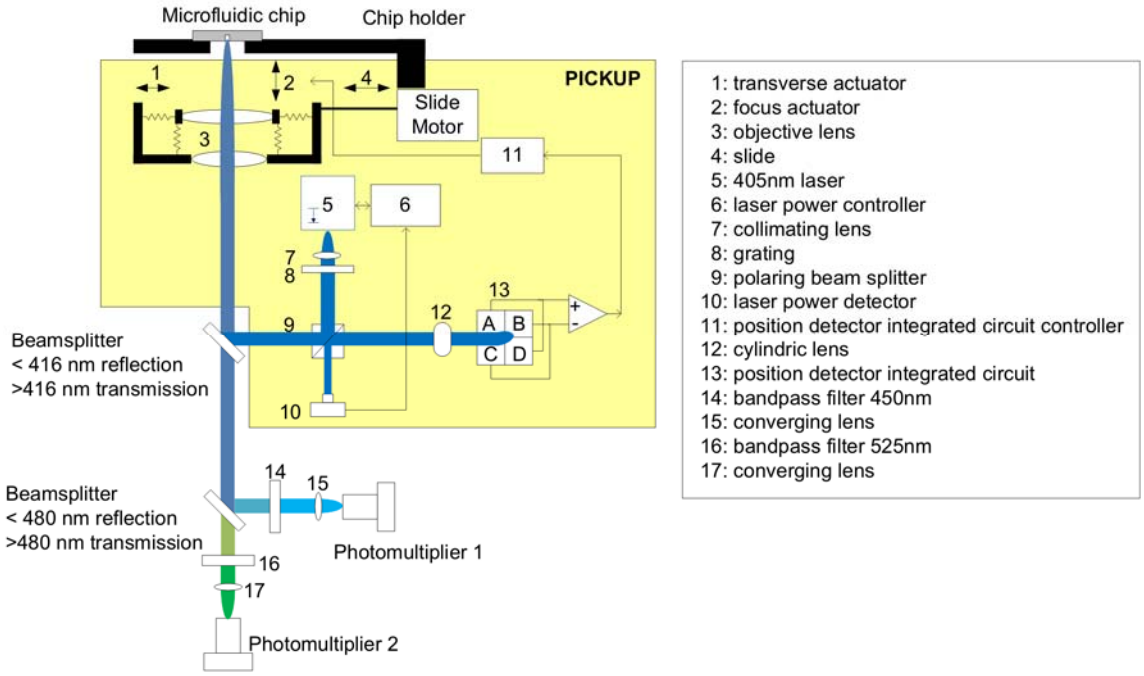

Figure 2: Schematic overview of the fluorescence detection system incorporating the HD-DVD pickup.

Table 1. The standards of the CD, DVD and HD-DVD format

	CD	DVD	HD-DVD
λ [nm]	780	660	405
NA	0.45	0.60	0.65
Resolution [µm]	1.06	0.67	0.38

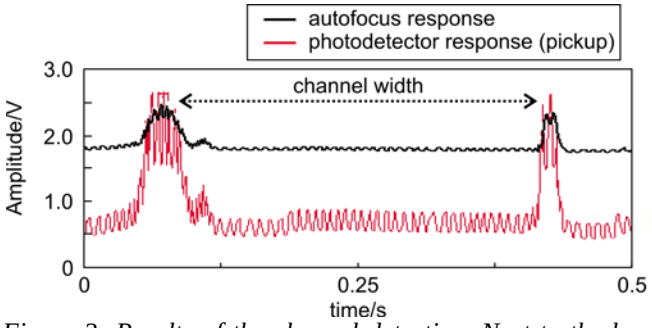

Figure 3: Results of the channel detection. Next to the border of the channel are reflective markers and these are detected (the peaks in the signals). The distance between the peaks corresponds to the channel width (100µm).

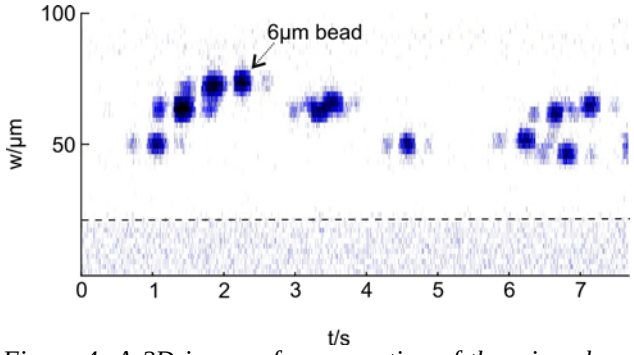

Figure 4: A 2D image of cross-section of the microchannel containing multiple 6µm fluorescent beads. The dashed line indicates the channel wall.

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