SINGLE CELL ANALYSIS BY NATIVE UV LASER INDUCED FLUORESCENCE DETECTION IN A PDMS MICROFLUIDIC CHIP

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ABSTRACT

We present an extension of our studies on single cell analysis with native label-free UV-LIF detection [1], in order to improve separation efficiency and detection sensitivity. We significantly reduced the background fluorescence by the fabrication of new PDMS-Quartz-Window-chips (PQW-chips) by at least 75 percent. Furthermore, the pH of the separation buffer was optimized for maximum signal intensity of the aromatic amino acid tryptophan. Based on these improvements we exemplarily present two single cell protein electropherograms from *Spodoptera frugiperda* (Sf9) insect cells with five times more and four times higher peaks as compared to our previous single cell studies [2].

Keywords: single cell, label-free UV-LIF detection, PDMS microfluidic chip, proteome

1. INTRODUCTION

Future single cell analysis allows individual expression studies, which are not limited by ensemble averaging effects from cell-cycle dependent states, the different and inhomogeneous cellular response to external stimuli, or the introduction of genomic and proteomic variability during cell proliferation. Experiments have shown that significant cellular phenotypes are only resolvable when studying single cells, emphasizing the importance of studying individual cells rather than heterogeneous populations [3]. For proteome research, effective, sensitive and quantitative separation and detection techniques are essential. Microfluidic devices have the potential to fulfil these requirements, which is impressively demonstrated by the transfer of proteome relevant separation techniques to the microfluidic format [4]. We could recently demonstrate a detection limit of 25 nM tryptophan (Trp) realised in a polydimethylsiloxane (PDMS) microfluidic device coupled to a sensitive label-free UV laser induced fluorescence (UV-LIF) detection system [2]. Thus we were able to perform single cell electropherograms on chip combined with UV-LIF detection [2].

2. EXPERIMENTAL

Chemicals and reagents were purchased from Sigma. Detailed chip design and operations are adapted from [1, 5] as well as an earlier proposed method for single cell analysis combining navigation and steering of single cells with optical tweezers, on-chip cell lysis and electrophoretic separation of proteins with subsequent LIF detection. For the production of PQW-chips, copper weights were used, which push down small fused silica windows onto the microchannel at the point of detection before the PDMS is cured. Fluorescence spectra were taken with an LS 45 luminescence spectrometer (PerkinElmer).

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3. RESULTS AND DISCUSSION

a) Chip enhancement: We significantly reduced the background fluorescence by at least 75 percent for native UV-LIF detection with the fabrication of new PQW-chips as compared to our previous PDMS-chips (Figure 2).

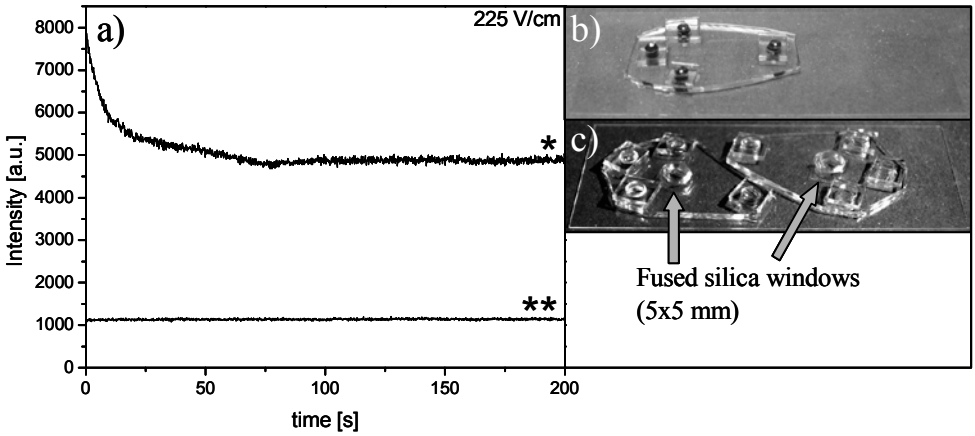

Figure 2. Fluorescence background reduction: (a) Fluorescence background of the microchannels filled with separation buffer (100 mM TRIS, 100 mM CHES, 4 % Pullulan, pH = 11,0), with 1000 µm pinhole and 500 µW laser power, λ_{ex} = 266 nm, (*) within the PDMS-chip and (**) within a PQW-chip. (b) Photograph of a PDMS-chip and c) two PQW-chips.

b) Increased signal intensity at basic pH: The investigation of the fluorescence intensity for the aromatic amino acid tryptophan in dependence of the separation buffer pH revealed an optimum pH value at ~11 for maximum intensity (Figure 3).

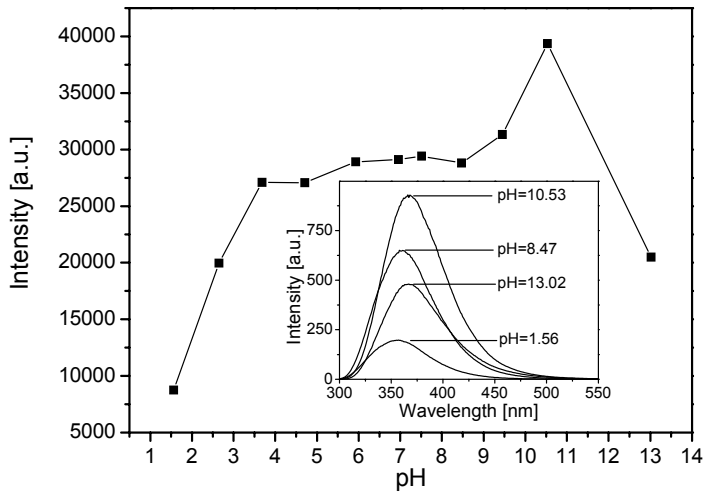

Figure 3. Increased signal intensity at basic pH: dependence of fluorescence intensity on pH for 10 mM tryptophan (Trp) solutions (in PBS), (λ_{ex} = 280 nm, λ_{det} = 335-385 nm). Inset: Selected emission spectra of the same Trp solutions from pH 1.6 to 13.0 (λ_{ex} = 280 nm).

c) Single cell electropherograms: Two single cell protein electropherograms from Sf9 cells are shown in Figure 4. These electropherograms exhibit five times more and four times higher peaks as compared to our previous single cell studies (total number of peaks: ~50-55) [2].

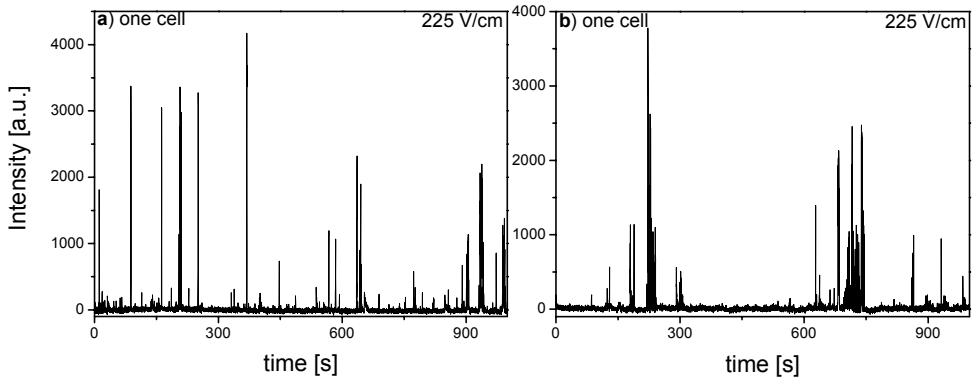

Figure 4. (a) Baseline corrected electropherogram from a single Sf9 cell with native UV-LIF detection (λ_{ex} = 266 nm). Separation buffer (100 mM TRIS, 100 mM CHES, 4 % Pullulan, pH = 11,0), 1000 µm pinhole and 500 µW laser power. The cells were electrically lysed with an electric field pulse (1800 V/cm, 50 ms). The channels in the PQW-chip were coated with F108. (b) Electropherogram of a single Sf9 cell with equal conditions as in (a).

4. CONCLUSIONS

The background fluorescence in native UV-LIF detection was significantly reduced by the production of the new PQW-chips. These PQW-chips integrate fused silica windows in the PDMS-microdevice at the point of detection. Thus, the advantages of normal PDMS-chips, like gas permeability and low costs were combined with those of full-body quartz glas chips, especially the high detection sensitivity. Further, the fluorescence intensity of the aromatic amino acid tryptophan, which is mainly responsible for the fluorescence signal in UV-LIF detection, could be raised by the use of a basic pH. With those amendments the UV-LIF detection was explicitly improved for single cell analysis. In the future, we will strive towards single cell fingerprinting with label-free UV-LIF detection.

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