A Valve-Controlled Microfluidic System for Rapid Two-Dimensional Electrophoresis

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OVERVIEW

(1) Rapid (350 s) two-dimensional electrophoretic separation of proteins in a PDMS microfluidic chip

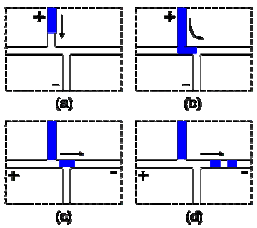
(2) Valves located at the intersection allow the simultaneous sample transfer between the 1-D single channel and 2-D channel array

(3) Scanning confocal method extends the size of both 1-D channel and 2-D array thus providing higher peak capacity for practical application

(4) Combining with the silicon electrospray tip facilitates the connection of electrophoresis and mass spectrometry

INTRODUCTION

Key point: transfer of analytes from 1-D to 2-D separations

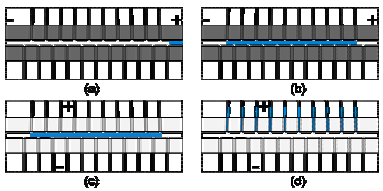


Fraction-transfer by running duty cycles

Time-consuming

Difficult control

Simple structure



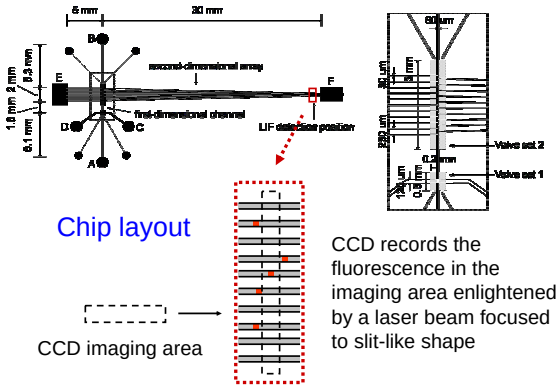
Simultaneous transfer

Rapidness

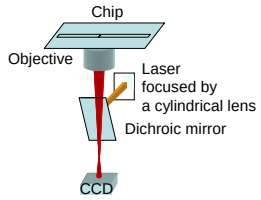
Easy control

Complicated structure

METHOD



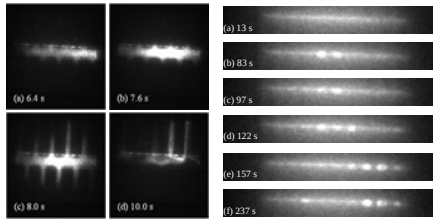
CCD records the fluorescence in the imaging area enlightened by a laser beam focused to slit-like shape



CCD imaging

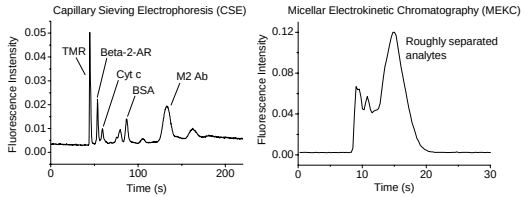
Laser-induced fluorescence for the detection of fluorescently labeled analytes

RESULT



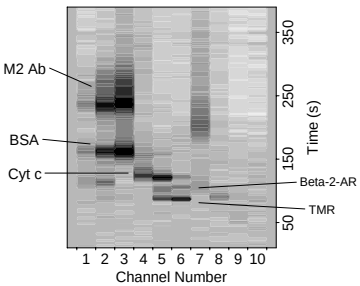
Simultaneous transfer of analytes at the intersection

CCD imaging of the detection area during separation



Independent 1-D and 2-D separations in a single channel

Beta-2-AR: beta-2-adrenergic receptor
Cyt c: cytochrome c
BSA: bovine serum albumin
M2 Ab: anti-FLAG M2 antibody



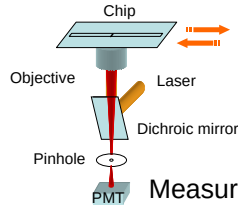
Separation of four proteins

Horizontal: MEKC
Vertical: CSE

However, the limited view field of microscope (10X objective lens) restricts the length of 1-D separation in a few millimeters, which results in inadequate 1-D resolution.

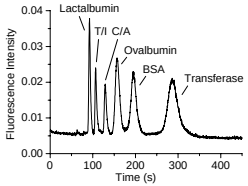
IMPROVEMENT

Scanning of the channels in 2-D array eliminates the restriction of the view field of microscope



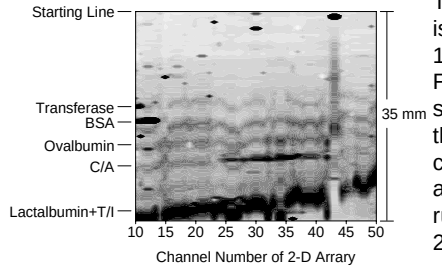
Using a stepper motor stage to fix the chip scan the channels with laser

Measure fluorescence with PMT



Separate a more complicated mixture of six proteins

T/I: trypsin inhibitor
C/A: carbonic anhydrase



The 2-D array is extended to 100 channels. For testing the scanning, fill the whole 1-D channel with analytes and run CSE in the 2-D array.

The bands tend to “contract” to the center of the channel during scanning which restricts the number of channels in the 2-D array.

FUTURE DIRECTION

(1) Connecting MEKC with CSE for two-dimensional separation

(2) Coupling with silicon ESI tip for mass spectrometry