PROTEOMICS-ON-A-CHIP FOR BIOMARKER DISCOVERY

Richard B.M. Schasfoort, Dietrich Kohlheyer, Stefan Schlautmann, Jan Eijkel and Albert van den Berg,
Biochip Group/BIOS, MESA+ Research Institute, The Netherlands.

ABSTRACT

In proteomics research still two-dimensional gel electrophoresis (2D-GE) is currently used for biomarker discovery. We applied free flow electrophoresis (FFE) separation technology combined with biomolecular interaction sensing using Surface Plasmon Resonance (SPR) imaging in an integrated proteomics-on-a-chip device as a proof of concept for biomarker discovery.

Keywords: Surface Plasmon Resonance imaging, Free Flow Electrophoresis, Proteomics, Label free detection

1. INTRODUCTION

Nowadays a dramatic shift in emphasis from mRNA profiling to proteomics occurs. Although 2D-GE is labor-intensive, slow, and prone to contamination, it is the highest resolution technique for the moment and is still the work-horse in proteomics research. In this paper we show features and benefits of a so-called "proteomics-on-a-chip" device for biomarker discovery based on electrophoresis principle and SPR phenomenon (see Fig. 1). An ideal technology should display the relevant biomarkers or specific binding partners from a cell extract. We developed a patented strategy to discover, categorize and identify potential biomarkers based on Free Flow Electrophoresis (FFE) and SPR imaging.

2. DEVICE DESIGN AND WORKING PRINCIPLE

Surface Plasmon Resonance (SPR) biosensors enable today's research to explore the kinetics of multiple biomolecular interactions (Fig. 1). In the Surface Plasmon Resonance imaging instrument for Biomolecular Interaction Sensing (iSPR-IBIS) from IBIS Technologies BV (Hengelo, NL) a scanning mirror is applied for detecting the so-called SPR-dips of many spots simultaneously. A microscopic view of the SPR reflection of the chip can be observed instantly and up to 500 biomolecular interactions can be

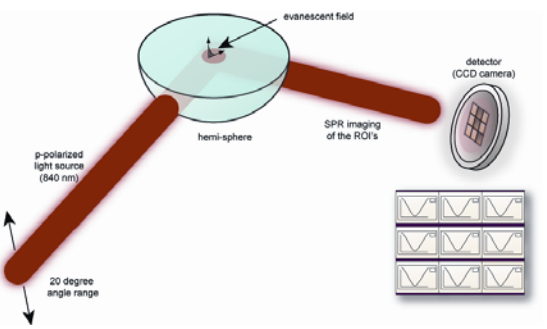


Fig.1. Schematic scanning SPR imaging setup; Biomolecular interactions at up to 500 "Regions of Interests" can be selected by the user and is being detected by a CCD camera.

followed in real-time and without using labels.

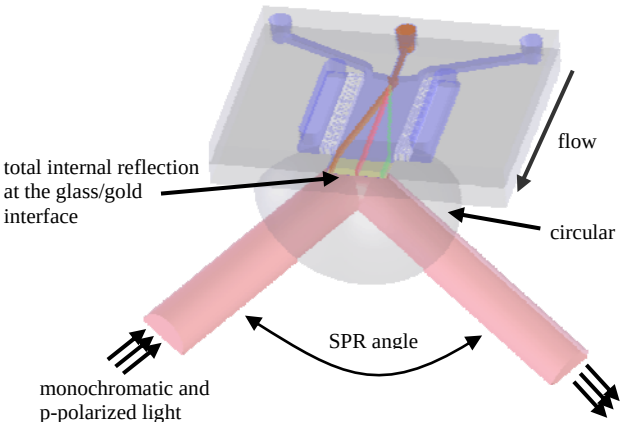
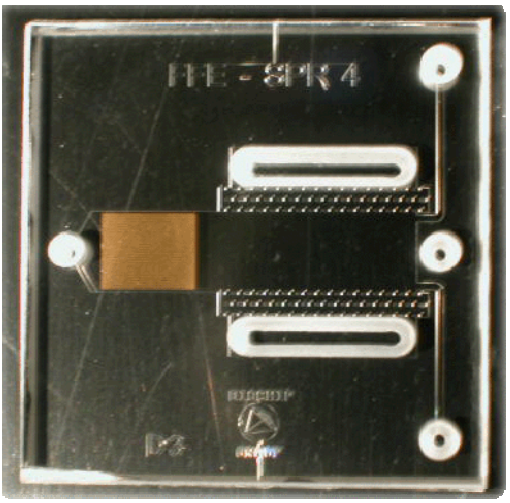


Fig.2 Principle of the "proteomics-on-a-chip" device. Protein sample is separated in the free flow electrophoreses section and guided over gold patches to be detected by Surface Plasmon Resonance imaging.

FFE is a continuous separation method, resulting in bands along a separation area and thus providing a continuous supply of separated components (see Fig. 2). Our approach is to sort and detect specific proteins and antibodies present in human blood serum which provide diagnostic information about e.g. autoimmune diseases. Instead of detection of fluorescent labeled components we use Surface Plasmon Resonance imaging which opens label-free and real-time detection of biomolecular interactions of FFE separated components.

3. MICROFABRICATION

A new FFE chip (see Fig. 3) has been fabricated, which contains a gold surface for SPR measurements. The IBIS instrument in scanning operation should detect the shift of the SPR-dips caused by biomolecular interactions in a special section of the free flow electrophoresis chip



A separated sample can be flown over the SPR gold patches and biomolecular interactions can be detected in real-time. A so-called dynamic blotting technique can be applied and biospecific interactions can be observed in real time and without using labels. The new approach should lead to biomarker discovery of specific diseases.

Fig. 3. FFE-SPR chip (20mm x 20mm) (left) At the right an image showing the SPR gold region inside the FFE chip

4. RESULTS AND DISCUSSION

The FFE-SPR chip was placed inside an IBIS SPR instrument and first preliminary results could be obtained as shown in Fig. 4. The photograph shows the gold region inside the chip with a centered sample stream. The surrounding water is in resonance, while the centered sample (Isopropanol) is out of resonance.

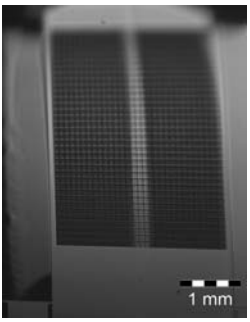
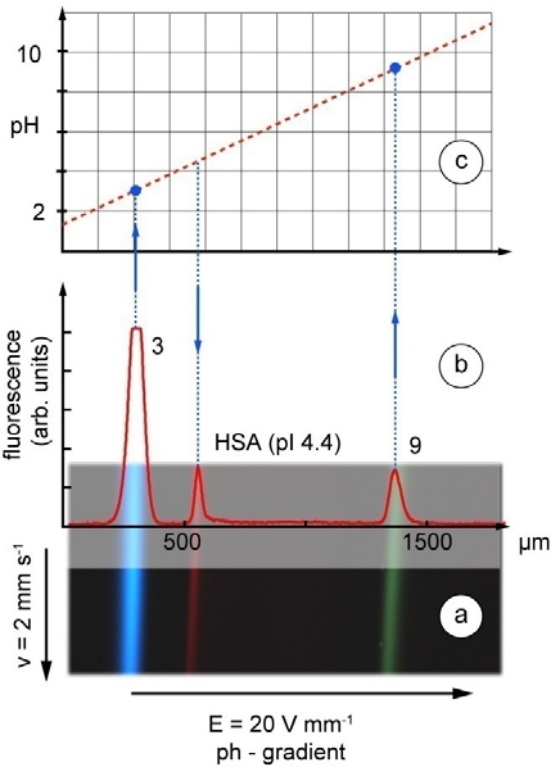


Fig.4 SPR image of the gold section of the IEF chip



An improved free-flow isoelectric focusing chip (FFEIEF) has been tested by Dietrich Kohlheyer with a set of fluorescent isoelectric focusing markers. For the first time, high resolution results could be obtained in such a microfluidic FFE device.

Fig. 5 FFIEF of two fluorescent IEF markers 3 and 9 and the protein HSA: The focusing of the low molecular weight standards results in wider bands compared to HSA. HSA focused at the pH value 4.4. Concentrations were: 330 µg ml^{-1} HSA, 80 µg ml^{-1} of IEF marker 3 und 9. The focusing residence time was 2.5 s.

5. CONCLUSIONS

It is definitely a trend that the integration of lab on a chip devices and SPR imaging instruments will further be developed for new applications in life sciences. Broadening the options of parallelization and assay implementation, including sample treatment on-a-chip as shown here by the FFE principle, would certainly contribute to the increase of the field of applications.

REFERENCES

[1] R.B.M. Schasfoort. Expert Rev. Proteomics 1(1), 123–132 (2004).
[2] D. Kohlheyer et al. , Lab on a Chip, 2006, 6, 374 – 380
[3] G.A.J. Besselink et. al., Electrophoresis, 25 2004