SARFUS: Direct detection of biomolecular interactions

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Abstract

Interaction between antibodies and peptide microspots are studied with Sarfus technique. Before incubation, measurement shows that the microspot thickness increases versus the concentration of the printed peptide solution. Incubation with antibodies results in the formation of a protein layer on the peptide microspot which was easily characterized using Sarfus technoqie.

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Introduction

Peptide microarrays provide an attractive technology to probe complex samples for the presence and/or function of biological analytes [1,2]. Optical [3], electrical [4] or mechanical [5] methods can be used for the detection of the captured molecules.

We show here that Sarfus allows a rapid imaging of protein films formed by the specific captured of arrayed peptides probes.

Experimental part

(See ref [6], [7], [8])

Fluorescence analysis

Fluorescence detection was performed at 532nm using a standard confocal microarray scanner.

Sarfus analysis

In this study, the topmost layer of the Surf substrates is SiO_2 ('Standard Surf'). Optical images are obtained on a LEICA DM4000 optical microscope and collected via a SONY 3CCD camera. The 2D images are treated with Sarfusoft (Nanolane software) and after calibration, 3D images are generated.

Results and discussion

Tag peptides derived from influenza hemagglutinin (HA) and *myc* protein (*myc*) were used in this study. They were printed on 2 nm-amine-modified SiO_2 substrates using a noncontact piezoelectric arrayer (figure 1) at different concentrations from 0.5 to 100µM (6 concentrations, 3 drops, 1nL overall). Finally, Standard Surf were washed and saturated with BSA.

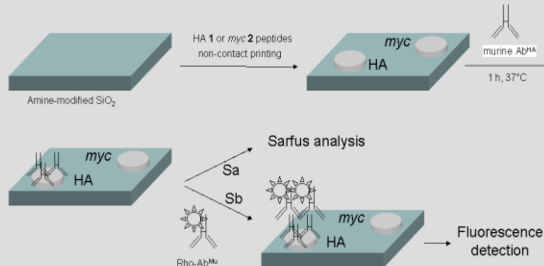


Figure 1: Antibody capture on HA and *myc* peptide microspot.

The method used to quantify the microspot thickness is illustrated on peptide HA (100µM) incubated with HA-antibodies (10µg/mL). Figure 2A show a typical Sarfus image. After calibration, false-color scale and 3D views (figures 2B & 2C) of the spot are obtained. Layer thickness was easily determined after profile extraction (figure 2D).

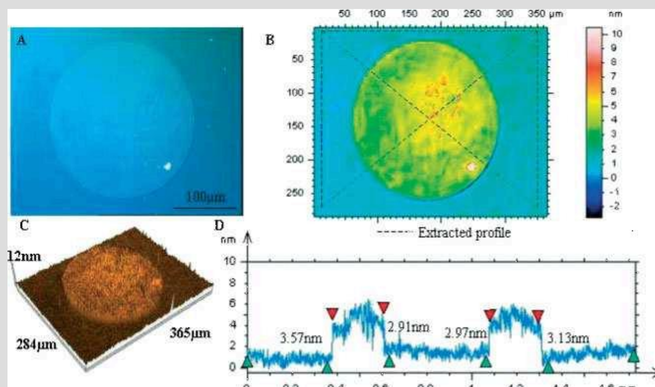
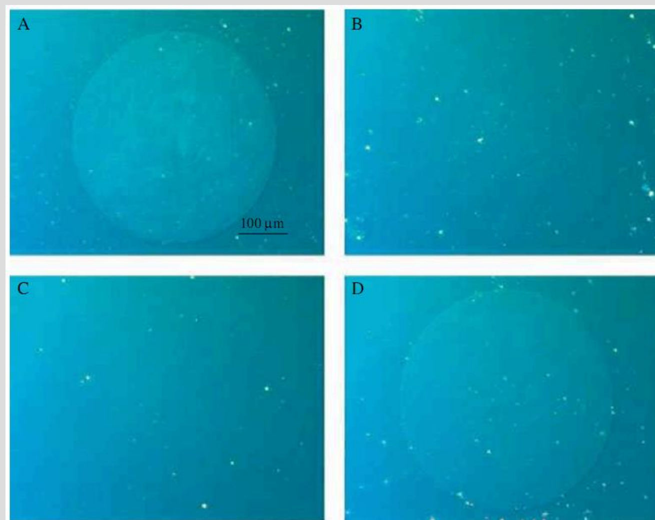


Figure 2: Microspot of peptide HA (100µM) printed on Standard Surf silanized with 3-aminopropyltrimethoxysilane and incubated (1h at 37°C) with murine anti-HA antibodies (10µg/ml). A) Sarfus image. B) False-color image. C) 3D view. D) Profile extraction (from dotted line on 2B).

We next examined the specificity of antibody layer formation. For this, incubations (1h at 37°C, 250µg/mL) with anti-HA or anti-*myc* murine antibodies in the presence of Tween® 20 and 2% Bovine Serum Albumine (BSA) then successive washes with PBS, water and ethanol were performed.

Anti-HA antibody on peptide HA (figure 3A) and anti-*myc* antibody on peptide *myc* (figure 3D) displayed a spot whereas no significant change could be observed for the HA microspot in the presence of anti-*myc* antibody (figure 3B) and for the *myc* microspots in the presence of the anti-HA antibody (figure 3C).



Figures 3: Sarfus images of HA peptides microarrays incubated with an anti-HA (5A) or anti-myc (5B) antibodies and of myc peptides microarrays incubated with an anti-HA (5C) or anti-myc (5D).

The effect of peptide or anti-HA antibody concentration on the thickness of the HA or *myc* microspots was examined (figure 4A). The peptides were printed at six different concentrations (from 0.5 to 100µM) and each antibody concentration (from 0.01 to 10µg/ml) was tested in triplicate. The spot heights (corresponding to the median and interquartile range of microspot heights) were found to be highly reproducible.

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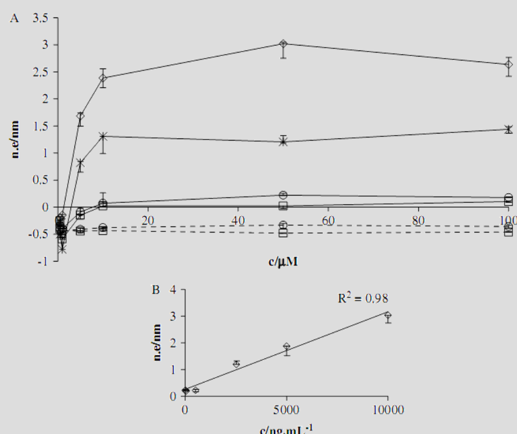
At low peptide concentration (1µM), the surface concentration of peptide/antibody complexes is probably not height enough to give a layer thicker than the surrounding BSA layer (figure 5). A plateau for a peptide concentration above 10µM is reached whatever the antibody concentration is. This is attributed to saturation of the surface by peptides above this concentration. In contrast to HA microspot, the altitudes of the peptide *myc* microspot in the presence of anti-HA antibodies at 10µg/mL were not significantly different from those obtained after washing with BSA/Tween 20®, illustrating the specificity of the antibody layer formation.



Figures 4: A. Evolution of spot thickness vs. peptide concentration (Peptide HA vs. anti-HA (continuous line) : ◊ 10µg/ml, × 2.5 µg/ml, ○ 0.01 µg/ml, □ not incubated; Peptide myc vs anti-HA (dotted line): ○ 10µg/ml, □ not incubated). B. Relationship between peptide HA microspot height and anti-HA antibody concentration.

One should note the linear relationship (r^2=0.98) observed between the anti-HA antibody concentration and HA (50µM) microspot height (figure 4B).

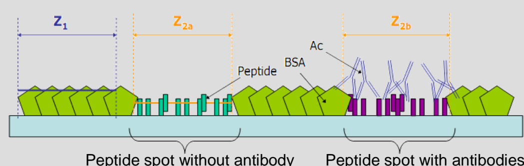


Figure 5: Sketch of the surface after BSA adsorption.

Conclusion

Sarfus allows the rapid imaging of protein layers formed by the specific binding of antibodies to array peptides probes. A linear relationship between the layer thickness and the antibody concentration was found.

Contribution/advantages of Sarfus

- Fast characterization technique for statistical results
- Non contact/ non labelling technique
- Field of view (from 60µm² to several mm²)
- Analysis at room temperature and atmospheric pressure

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