Journal of
Proteomics & Bioinformatics
ISSN: 0974-276X

Sjöberg et al., J Proteomics Bioinform 2012, 5:8
DOI: 10.4172/jpb.1000233

Research Article Open Access

Biosensor Based Protein Profiling on Reverse Phase Serum Microarray

Ronald Sjöberg[1], Lennart Hammarström[2] and Peter Nilsson[1]*

[1]*SciLifeLab Stockholm, School of Biotechnology, KTH – Royal Institute of Technology, Box 1031, SE-17121 Solna, Sweden*
[2]*Division of Clinical Immunology, Karolinska Institute, Karolinska University Hospital, Huddinge, SE-14186 Stockholm, Sweden*

Abstract

The reverse phase serum microarray format enables multi-parallel and simultaneous analysis of literally thousands of samples, a feature which is of uttermost importance for protein profiling of clinical samples. We have here screened 2400 serum samples for their potential IgA deficiency by using a fluorescence based reverse phase serum microarray platform and a biosensor based label-free microarray platform for verification and also compared our microarray-results to clinical routine ELISA. We have been able to identify possible IgA-deficiencies and to show the suitability of our microarray-platforms for large-scale screening of clinical serum samples.

The two microarray methods show reproducibility and correlation towards each other and low variation between replicates within each platform. Both of the microarray platforms show less agreement towards ELISA. The fluorescence based microarray method has been shown to be applicable for large-scale screening of clinically important serum samples for detection of possibly IgA-deficient patients. Furthermore, it was found that the microarray based biosensor method could be used for determining the relative differences in concentration of IgA between the samples.

Keywords: Reverse phase serum microarray; Protein profiling and screening; SPR; Biosensor; Label-free

Introduction

The reverse phase microarray format enables multi-parallel and simultaneous analysis of literally thousands of samples, a feature which is of uttermost importance for protein profiling of clinical samples and which is also often a limiting factor. Other microarray based technology platforms might have the capacity to profile large numbers of targets and analytes, but is usually restricted in the multiplexing dimension of samples. In the reverse phase microarray platform the samples are immobilised on the substrate in an array configuration and the affinity reagents are subsequently applied as detection reagents. This enables large numbers of samples being profiled for one or a few targets simultaneously under the same experimental conditions while using low sample volumes. On the other hand, in the forward phase array where the affinity reagents, antibodies or other binding molecules, are immobilised on the functionalised surface, the samples containing the antigens are then applied to the array. This allows for the simultaneous analysis of a few samples towards a high number of targets.

The majority of the described applications of reverse phase microarrays have so far mainly involved various forms of either cell lysates [1,2] or tissue lysates [3,4]. We have previously described the application of serum microarrays. This have been in the context of analysis of IgA levels in 2000 patients [5], screening for IgA deficiency in 5000 children [6] and screening for C3 deficiency in newborns with spotted extracts from paper dried blood spot samples [7].

There is an inherent sensitivity issue with reverse phase serum microarrays. This limitation arises due to the large dynamic range of proteins present in blood where many proteins are present in low pg/ml and thereby fM [8]. Proteins that are present in such low concentrations in whole blood will be represented by only a few molecules if the total sample volume is in the sub-nano litre scale spotted in the array which greatly impedes sensitive measurements. Efforts are needed and ongoing to develop methodologies and techniques for increased detectability utilizing various types of signal amplification as for example reviewed by Nong *et al.* [9] for DNA-based technologies. It is although still in its current direct setup a platform suitable for detecting medium to highly abundant proteins while low abundant proteins will be challenging to detect reliably. Within the IgA profiling the limits of detection has been found to be in the high ng/ml to the low µg/ml range [5].

When conducting large-scale antibody based protein profiling experiments and screening of samples, confirmation and verifications of early indications are of fundamental importance. Initial technical verifications usually involve repeated measurements and various replicates and often before looking into different and most often also larger sample sets, there is also a value to confirm screening results with alternative methods and technologies. One such alternative is to use a biosensor based label-free platform avoiding the need for a secondary reagent since no labelled detection molecule is utilized. Measuring a property of a binding event itself also allows for real-time measurement and extraction of reaction kinetics of the event. Label-free methods can be based on, the polarization state of reflected light [10,11], interference fringes [12-14], or a number of other properties [15-20]. Surface plasmon resonance (SPR) sensors are based on the changes in refractive index that are produced by the interaction between molecules immobilised on a detection surface and molecules in a liquid sample flowing over the detection surface [21-25].

The majority of currently available biosensor instruments does not allow for multi-parallel analysis in an array-based format, but there are some platforms that can provide the necessary sample throughput [26].

***Corresponding author:** Peter Nilsson, SciLifeLab Stockholm, School of Biotechnology, KTH – Royal Institute of Technology, Box 1031, SE-17121 Solna, Sweden, E-mail: peter.nilsson@scilifelab.se

Received August 02, 2012; **Accepted** August 13, 2012; **Published** August 15, 2012

Citation: Sjöberg R, Hammarström L, Nilsson P (2012) Biosensor Based Protein Profiling on Reverse Phase Serum Microarray. J Proteomics Bioinform 5: 185-189. doi:10.4172/jpb.1000233

Copyright: © 2012 Sjöberg R, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

This makes them suitable as alternative platforms for confirmation of results obtained from large-scale screenings on reverse phase fluorescent microarrays.

We have previously developed a reverse phase microarray platform for large-scale simultaneous probing of serum samples [5]. We have now applied this platform to 2423 serum samples from children in order investigate the feasibility of using this platform in large-scale screening of children for detection of IgA-deficiency in combination with a biosensor based microarray platform for validation. A subset of those samples with indications of being IgA deficient were transferred to a SPR-based platform in order to confirm the results, we have also compared the two microarray platforms to the commonly used ELISA.

Materials and Methods

Experimental setup

2423 serum samples from children were printed on glass slides and analysed for their IgA content. 182 of those samples were reprinted on glass slides and reanalysed as well as printed on a sensor chip for SPR-analysis. This selected set consisted of 28 samples with a concentration of IgA of 0.3 mg/ml or less, 100 samples that showed large discrepancies between ELISA and fluorescence microarrays, and a 54 sample set that were randomly chosen from the sample pool. A comparison between replicates and methods were performed as well as a comparison with ELISA-values for those samples.

Fluorescence based microarray

The serum samples were diluted 1:10 PBS with 0.5% Tween20, loaded onto 384 well plates in volumes of 30 µl/well (Genetix) and printed in duplicate (in 14 identical blocks) on Corning Epoxide slides (Corning) using a non-contact printing robot (Nano-plotter 2.0, Gesim). The slides were incubated in a humidity chamber (75%) for 16 hours at 20°C and blocked with Super Block solution (Pierce) using an air-brush pistol. Polyclonal rabbit anti-human IgA antibodies (DAKO) were added at a concentration of 46 ng/ml. Alexa Fluor 555 goat-anti-rabbit IgG (Molecular Probes) was used as a secondary antibody at a concentration of 33 ng/ml. The slides were scanned in a G2565BA array scanner (Agilent) with the photomultiplier tube set to 100% for both channels and the scan resolution set to 10 µm. The resulting images were analyzed with GenePix-Pro 5.1 (Molecular Devices) using non-circular feature alignment.

Biosensor based label-free microarray

A total of 200 samples consisting of 182 serum samples with known IgA levels and 18 control samples where diluted 1:10 in 0.5% Tween20 in 1x PBS and printed in duplicates with a non-contact microarray printer (Nanoplotter2, GeSiM).

Blocking of the detection surface was conducted in the FlexChip instrument (GE Healthcare,Biacore Systems) by filling the flowcell with 0.1% Tween 20 in 1xPBS with 10% Bovine Serum Albumin (BSA Cohn fraction V, protease free, Saveen Werner) five times for five minutes each. A baseline was established by flowing the running buffer (0.1% Tween 20 in 1xPBS) through the flow cell for ten minutes before the first antibody injection.

As a negative control anti-rabbit IgG antibody (Jackson ImmunoReasearch) was used, anti-human HSA antibody (Jackson ImmunoReasearch) was used as a positive control, anti-human IgG antibody (DakoCytomation) was used for verification of normal IgG levels in the samples and a blank sample consisting of 0.1% Tween 20 in 1xPBS was used to verify that no unspecific binding occurred due to the dilution buffer.

The antibodies where diluted in running buffer to final concentrations of 50 µg/ml in separate sample tubes and sequentially recirculated through the flowcell for five minutes each with a five minute disassociation phase in between every injection. The order of injection was; anti-rabbit IgG, anti-human IgA, anti-human C3, anti-human IgG, anti-human HSA, 0.1% Tween 20 in PBS (Figure3).

The resulting binding curves yields full kinetic data for the binding by extracting two report points, binding early and binding late, from the association phase and two report points, stability early and stability late from the dissociation phase. In this work only the late stability values were used in the comparison between platforms. This was repeated on two detection chips to yield a total of four binding curves for each serum sample.

ELISA

Total serum IgA levels were determined by sandwich ELISA using polyclonal rabbit anti-human IgA antibodies (DAKO) and alkaline phosphatase-conjugated rabbit anti-human serum IgA antibodies (Jackson ImmunoResearch), added at a concentration of 1.2 µg/ml and 0.6 µg/ml respectively. Polystyrene plates (Corning) were coated over night at room temperature with 100 µl per well of the primary antibody diluted in carbonate bicarbonate buffer (0.05M). The plates were washed four times with phosphate-buffered saline (PBS) with 0.5% Tween 20 between the incubations. The samples were three-fold serially diluted in PBS with 0.5% Tween 20. All samples were titrated against a six-fold serially diluted standard, ranging from 3.1 ng/ml to 100 ng/ml. The samples, the standard dilutions and a blank (PBS with 0.5% Tween 20) were added in duplicate (100 µl/well) and incubated over night at room temperature. The alkaline phosphatase-conjugated antibodies were added (100 µl/well) and incubated for 2 hours in room temperature. p-Nitrophenyl phosphate (Sigma-Aldrich) was added and the absorbance was read on a Vmax microplate reader (Molecular Devices). A mean concentration was obtained for each sample using Deltasoft JV 1.8 (Biometallics). In addition, total serum IgG levels were determined in the individuals with IgA deficiency using sandwich ELISA. Polyclonal rabbit anti-human IgG antibodies (DAKO) and alkaline phosphatase-conjugated polyclonal rabbit anti-human IgG antibodies (DAKO) were added at a concentration of 0.6 µg/ml and 1.1 µg/ml respectively. The same protocol as for determination of serum IgA was followed.

Data analysis and normalisation

For both array platforms a simple global median normalization was performed in order to correct for experimental artifacts. This was done by multiplying each data point with the ratio between the median of the individual slide and the median of all slides. The median intensity of each spot was averaged based on replicates and the correlation between the microarrays and ELISA was calculated. A scaling factor was calculated between the data from the arrays and the data from ELISA as the ratio between the median of the array data and the median of the ELISA data and applied to the array data for scaling to mg/ml for comparison with ELISA values. The coefficient of variation and Pearson correlation were calculated between replicate ELISA experiments, replicate microarray printings and between replicate SPR-sensor chips. All statistical analysis and normalisation were done using R, a language for data analysis and graphics (www.r-project.com).

Results and Discussion

We have in a high-throughput fashion screened 2400 serum samples from children to identify possible IgA-deficiencies with the goal of utilize a fluorescence-based microarray platform as a tool for large-scale screening of clinically relevant samples and to use a biosensor based label-free reverse phase microarray platform for validation. We have confirmed the results on a subset of 182 samples consisting of samples that were identified as IgA-deficient on ELISA and samples that showed large discrepancy between the two platforms. This was done using a microarray-based biosensor with label-free surface plasmon resonance detection and the results from both microarray-based platforms were compared with results from ELISA in order to compare the results obtained from the microarray platforms to a clinically common platform and to investigate the suitability of the SPR-based platform for detecting IgA-deficiencies (Figure 1).

Fluorescence microarrays

Printing the serum samples yielded spots with uniformly homogenous morphology. The correlation between replicate slides and within slides were both r=0.98 and between printings r=0.90. This high correlation between technical replicates show that the protocol used enables consistent generation of high quality sample spots which is necessary in order to ensure that sufficient precision in determining deficient samples is achieved and to minimise the risk of producing false negatives (Figures 2A-2C). Separation between deficient samples and non-deficient samples were achieved to a satisfactory degree making it possible to identify possible deficiency samples that can be further analysed in order to validate their lack of IgA (Figure 4D).

This means that it is possible to use this protocol for high-throughput screening of serum samples for determining which samples show low IgA-levels that could be a result of IgA-deficiency in the patient. This would best be achieved by first performing a large initial screening of samples in order to investigate the relative amount of IgA and then reanalyse the lowest range to confirm possible deficiencies. The initial large-scale screening can be performed upon thousands of samples simultaneously and thus minimise the amount of time and money needed to perform each sample analysis. This kind of screening is best performed in a microarray format with fluorescence labelling due to the possibility to easily print and analyse samples in the tens of thousands. The subsequent reanalysis of the lowest ranged samples is more suitable to be performed in a label-free format due to its lack of complicated and time-consuming blocking procedures and secondary antibody binding steps. If needed a final analysis of the identified deficiencies can be analysed on ELISA in order to determine their exact IgA-concentration.

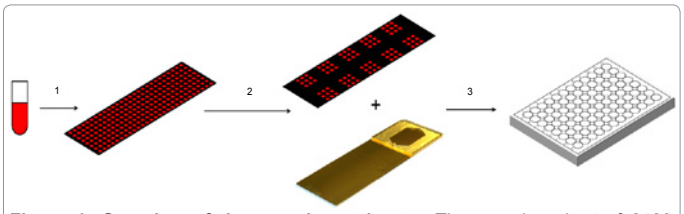

Figure 1: Overview of the experimental setup. The sample cohort of 2423 serum samples were initially screened in a high-throughput manner on fluorescence arrays (1), 182 samples were then re-printed and re-analysed on fluorescence arrays as well as on the label-free platform (2).These datasets were then compared with ELISA-values for evaluation purposes (3).

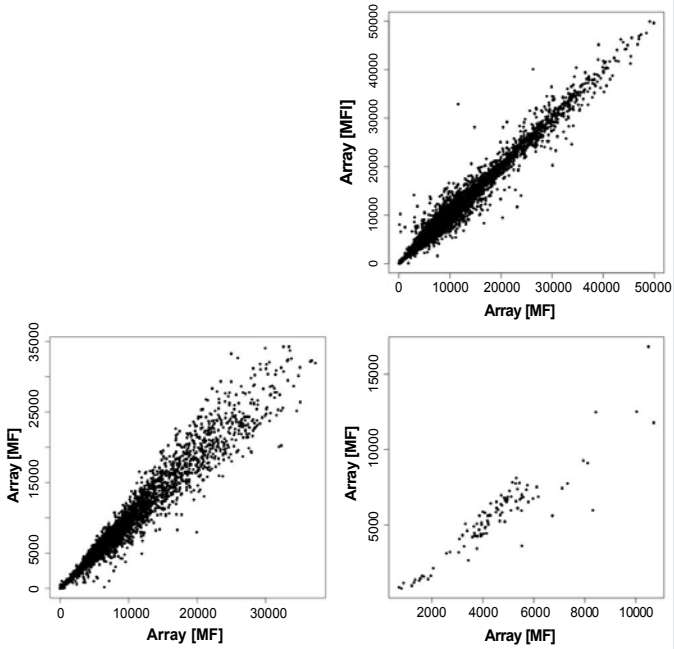

Figure 2: A) Quality of spot reproducibility within slides. Plotting the spot replicates within each slide in a correlation plot with spot replicate one on the x-axis and spot replicate two on the y-axis showed that the spot replicates have a good correlation towards each other (R = 0.98) indicating that the spotting procedure is robust and have good reproducibility and that few spot replicates are needed in order to get reliable results. **B) Quality of spot reproducibility between slides.** Plotting two slide replicates against each other show good reproducibility between the replicates with a good correlation (R = 0.98) albeit with lower correlation for samples with higher contents of target protein. **C) Quality of spot reproducibility between separate prints.** 182 samples were reprinted and reanalysed on fluorescent arrays and results from the replicate printings were plotted against each other. This showed comparable results with good correlation (R = 0.9) between printings which indicates that single printings would be sufficient for obtaining data.

SPR-microarrays

We printed 182 serum samples with known IgA levels and 18 control samples in duplicates forming a total of 400 spots resulting in 400 simultaneous binding curves per injection (Figure 3). Using duplicate chips and six injections we obtained a total of 4800 binding curves. The duplicate positive binding curves showed a correlation between chip replicates of r=0.98as well as sufficient separation between deficient and non-deficient samples confirming the results from the fluorescence based analysis. Interrogating the immobilised samples with an anti-IgG antibody showed that there were no correlation between the IgA-levels and the general IgG-levels ensuring that the results are specific to the IgA-levels (Figure3C). We have chosen to use the stability latepoint of the dissociation part of the binding curve for measuring the IgA-levels but the results were similar for the binding early, binding late and stability early measurements meaning that either of these points on the binding curve could be used for determining the relative amount of IgA.

Comparison with ELISA

When comparing the two microarray-based platforms to ELISA the results from the microarray-based platforms proved to be more consistent with each other than with the results from ELISA (Figures 4A– 4C).ELISA show lower correlation between replicates (r=0.57 vs. r=0.90 for the fluorescence-based platform and r=0.98 for the SPR-

based platform) which implicates overall better robustness for the microarray platforms. This is of high importance in a clinical setting since it would mean that fewer technical replicates are needed to minimise the risk of false positives or false negatives.

This inconsistency between the two microarray methods and ELISA becomes more apparent at increasing IgA concentration which might mean that ELISA lacks accuracy at high concentrations of the target molecule. Although when using ELISA to identify samples with IgA deficiency, defined as serum IgA levels below 0.07 mg/ml, and plotting the two groups for all three platforms they all show good separation between the deficient samples and the normal samples. The lower correlation between replicates as well as the low correlation between the two microarray platforms and ELISA (r=0.66 for ELISA vs. fluorescence based arrays and r=0.76 for ELISA vs. SPR-based arrays might be an effect of the low throughput of ELISA that requires the samples to be analysed in small batches over a long period of time while the high-throughput array-platforms allow analysis of large sample cohorts under the same experimental conditions. It could otherwise have been expected that ELISA and the fluorescence-based microarray platform would have behaved more similar to each other than to the SPR-platform due to the similar setup using primary and secondary labelled binders, while the SPR-platform only need the primary binder and therefore should be better suited for analysing complex samples.

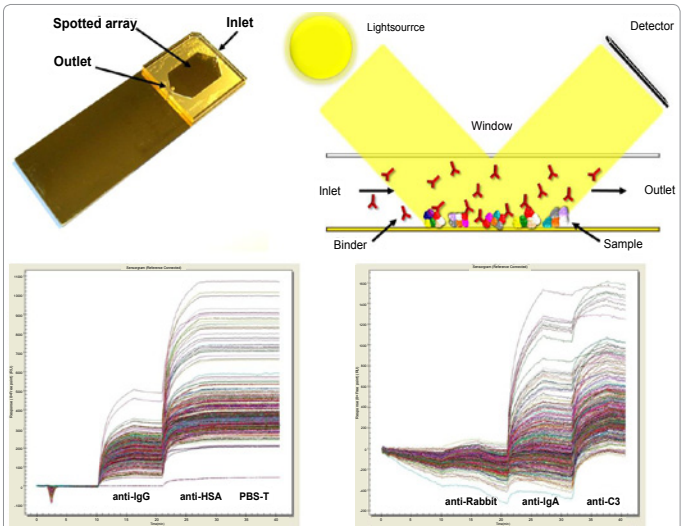

Figure 3: A) The SPR-sensor chip and its flow-cell. The image shows an SPR-sensor chip with attached window gasket. Inlet and outlet is part of the gasket which is attached after spotting of the array. The area available for spotting the array is a square of 1 x 1 cm. **B) Schematic illustration of the SPR-sensor chip flowcell.** A schematic illustration of the flowcell and its detection system is shown where anti-IgA antibodies are circulated through the flowcell and as binding to, and dissociation from, the immobilised samples occur the change of refraction is monitored by the detector and recorded. **3C: Recorded binding curves from the first set of three analytes.** A total of 400 simultaneously recorded binding curves from label free analysis with anti-IgG, anti-HSA, and running buffer. Both anti-IgG and the positive control anti-HSA show strong binding to all spots except the sample buffer spot. Results from anti-IgG show no correlation to results from anti-IgA indicating that the IgA-deficient samples contains normal IgG levels. Anti-HSA functions as a positive control and indicates that all spots are present. **D) Recorded binding corves from the second set of three analytes.** Binding curves from label free analysis with negative control (anti-rabbit), anti-IgA, and anti-C3 as a second positive control. They show minimal binding of the negative control to all spots and varying binding from anti-IgA, which is to be expected. The Spearman correlation coefficient between replicate chips was R = 0.98 which implies that single chip would be enough in order to validate the results from the fluorescent arrays.

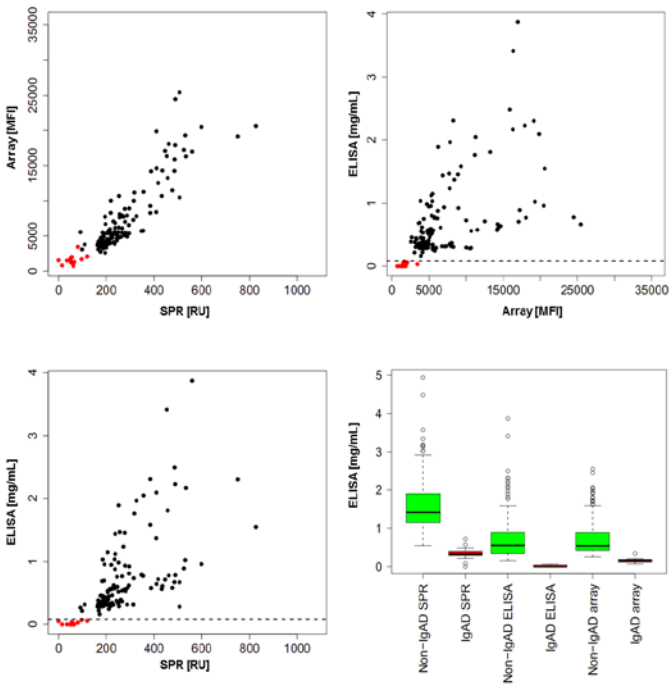

Figure 4: A) Scatterplot of the label free SPR-arrays vs. fluorescence arrays. Plotting the two array-based methods show acceptable agreement between methods with a correlation of r = 0.89. **B) Scatterplot of the fluorescence arrays vs. ELISA.** Plotting the fluorescence arrays against ELISA show less agreement with a lower correlation then between the array-based platforms (r = 0.66). **C) Scatterplot of the SPR-based arrays vs. ELISA.** The SPR-based arrays and ELISA also show less agreement between platforms then the two array-based platforms (r = 0.76). **D) Separation of the deficient samples from non-deficient.** Boxplots of the two groups of samples show satisfactory separation between groups for the label-free detection method as well.

Conclusions

We have in this work analysed 2400 serum samples in a large-scale screening on a microarray platform based on fluorescent labelling and confirmed the results on a microarray platform based on surface plasmon resonance. Sufficient separation between deficient and non-deficient samples was achieved for identification of deficient samples even though no depletion of the samples was performed before hand. No correlation between IgA-levels and general IgG-levels could be found meaning that identified IgA-deficiency samples are not suffering from general IgG-deficiency.

For benchmarking purposes we have compared the two microarray platforms to ELISA. We found that the fluorescently labelled and the SPR-based microarray platforms show higher correlation between replicates than ELISA and confirm each other with better correlation towards each other than towards ELISA. ELISA show increasing disagreement with the microarray-based methods at higher concentrations of the target and show a low reproducibility of results which imply a lower accuracy for ELISA when analysing for IgA in serum.

This means that utilising a microarray-based platform with fluorescence-based detection appears highly suitable for screening large cohorts of samples to determine their relative concentration of IgA. The set of samples that show the lowest relative concentration can then be reanalysed on a SPR-based method to screen that smaller subset of samples in an effective way of validating possible IgA-deficiencies.

Acknowledgments

We like to thank Magdalena Janzi at Karolinska Institutet for excellent technical assistance, Björn Persson and Stefan Lövås at GE Healthcare and Jochen Schwenk at SciLifeLab for fruitful discussions. This study was supported by the ProNova VINN Excellence Centre for Protein Technology (VINNOVA, Swedish Governmental Agency for Innovation Systems) and by grants from the Knut and Alice Wallenberg Foundation.

References

1. Sevecka M, Wolf-Yadlin A, MacBeath G (2011) Lysate microarrays enable high-throughput, quantitative investigations of cellular signaling. Mol Cell Proteomics 10: M110 005363.
2. Nishizuka S, Charboneau L, Young L, Major S, Reinhold WC, et al. (2003) Proteomic profiling of the NCI-60 cancer cell lines using new high-density reverse-phase lysate microarrays. Proc Natl Acad Sci U S A 100: 14229-14234.
3. VanMeter AJ, Rodriguez AS, Bowman ED, Jen J, Harris CC, et al. (2008) Laser capture microdissection and protein microarray analysis of human non-small cell lung cancer: differential epidermal growth factor receptor (EGPR) phosphorylation events associated with mutated EGFR compared with wild type. Mol Cell Proteomics 7: 1902-1924.
4. Gulmann C, Sheehan KM, Conroy RM, Wulfkuhle JD, Espina V, et al. (2009) Quantitative cell signalling analysis reveals down-regulation of MAPK pathway activation in colorectal cancer. J Pathol 218: 514-519.
5. Janzi M, Odling J, Pan-Hammarstrom Q, Sundberg M, Lundeberg J, et al. (2005) Serum microarrays for large scale screening of protein levels. Mol Cell Proteomics 4: 1942-1947.
6. Janzi M, Kull I, Sjoberg R, Wan J, Melen E, et al. (2009) Selective IgA deficiency in early life: association to infections and allergic diseases during childhood. Clin Immunol 133: 78-85.
7. Janzi M, Sjoberg R, Wan J, Fischler B, von Dobeln U, et al. (2009) Screening for C3 deficiency in newborns using microarrays. PLoS One 4: e5321.
8. Jacobs JM, Adkins JN, Qian WJ, Liu T, Shen Y, et al. (2005) Utilizing human blood plasma for proteomic biomarker discovery. J Proteome Res 4: 1073-1085.
9. Nong RY, Gu J, Darmanis S, Kamali-Moghaddam M, Landegren U (2012) DNA-assisted protein detection technologies. Expert Rev Proteomics 9: 21-32.
10. Klenkar G, Liedberg B (2008) A microarray chip for label-free detection of narcotics. Anal Bioanal Chem 391: 1679-1688.
11. Fei YY, Landry JP, Sun YS, Zhu XD, Luo JT, et al. (2008) A novel high-throughput scanning microscope for label-free detection of protein and small-molecule chemical microarrays. Rev Sci Instrum 79: 013708.
12. Ozkumur E, Needham JW, Bergstein DA, Gonzalez R, Cabodi M, et al. (2008) Label-free and dynamic detection of biomolecular interactions for high-throughput microarray applications. Proc Natl Acad Sci U S A 105: 7988-7992.
13. Wang X, Zhao M, Nolte DD (2008) Area-scaling of interferometric and fluorescent detection of protein on antibody microarrays. Biosens Bioelectron 24: 987-993.
14. Mace CR, Striemer CC, Miller BL (2008) Detection of human proteins using arrayed imaging reflectometry. Biosens Bioelectron 24: 334-337.
15. Sinensky AK, Belcher AM (2007) Label-free and high-resolution protein//DNA nanoarray analysis using Kelvin probe force microscopy. Nat Nanotechnol 2: 653-659.
16. Lynch M, Mosher C, Huff J, Nettikadan S, Johnson J, et al. (2004) Functional protein nanoarrays for biomarker profiling. Proteomics 4: 1695-1702.
17. Zheng G, Patolsky F, Cui Y, Wang WU, Lieber CM (2005) Multiplexed electrical detection of cancer markers with nanowire sensor arrays. Nat Biotechnol 23: 1294-1301.
18. Torres FE, Kuhn P, De Bruyker D, Bell AG, Wolkin MV, et al. (2004) Enthalpy arrays. Proc Natl Acad Sci U S A 101: 9517-9522.
19. Huber F, Lang HP, Hegner M, Despont M, Drechsler U, et al. (2008) Analyzing refractive index changes and differential bending in microcantilever arrays. Rev Sci Instrum 79: 086110.
20. Walter JG, Kokpinar O, Friehs K, Stahl F, Scheper T (2008) Systematic investigation of optimal aptamer immobilization for protein-microarray applications. Anal Chem 80: 7372-7378.
21. Homola J (2003) Present and future of surface plasmon resonance biosensors. Anal Bioanal Chem 377: 528-539.
22. van Vuuren BJ, Read T, Olkhov RV, Shaw AM (2010) Human serum albumin interference on plasmon-based immunokinetic assay for antibody screening in model blood sera. Anal Biochem 405: 114-120.
23. de Boer AR, Hokke CH, Deelder AM, Wuhrer M (2008) Serum antibody screening by surface plasmon resonance using a natural glycan microarray. Glycoconj J 25: 75-84.
24. Xinglong Y, Dingxin W, Xing W, Xiang D, Wei L, et al. (2005) A surface plasmon resonance imaging interferometry for protein micro-array detection. Sensors and Actuators B: Chemical 108: 765-771.
25. Lindquist NC, Lesuffleur A, Im H, Oh SH (2009) Sub-micron resolution surface plasmon resonance imaging enabled by nanohole arrays with surrounding Bragg mirrors for enhanced sensitivity and isolation. Lab Chip 9: 382-387.
26. Scarano S, Mascini M, Turner AP, Minunni M (2010) Surface plasmon resonance imaging for affinity-based biosensors. Biosens Bioelectron 25: 957-966.