ELSEVIER

Analytica Chimica Acta 411 (2000) 1–11

ANALYTICA CHIMICA ACTA

www.elsevier.com/locate/aca

Disc-based immunoassay microarrays

Horacio Kido, Angel Maquieira, Bruce D. Hammock*

Department of Entomology, University of California, Davis, CA 95616, USA

Received 26 July 1999; received in revised form 16 December 1999; accepted 6 January 2000

Abstract

Microarray technology as applied to areas that include genomics, diagnostics, environmental, and drug discovery, is an interesting research topic for which different chip-based devices have been developed. As an alternative, we have explored the principle of compact disc-based microarrays. This new methodology successfully combined high-density microarrays applied via a piezoelectric inkjet applicator with circular indexing on a polycarbonate disc. As a demonstration of the principle, we ran competitive inhibition immunoassays for hydroxyatrazine, carbaryl, and molinate. The resulting microspots were approximately 75 micrometers in diameter and were visualized by using an antibody labeled with a fluorescent tag and a commercially available fluorescence scanner. The results of this work suggest that compact disc-based microarray technology can give qualitative and quantitative results and has potential for simultaneous multianalyte analyses.

Keywords: Microarray; Immunoassay; Compact disc; Diagnostics

1. Introduction

Since the first automatic analyzers were introduced into laboratories 30 years ago [1], great advances in miniaturization and automation of analytical methods have been made [2,3]. The most prominent example of these advances is the DNA microchip, which combines sophisticated lithographic immobilization techniques used in the semiconductor manufacturing industry with oligonucleotide synthesis to form high-density microarrays on silicon chips [4–6]. In general these chips are approximately 1.5 cm × 1.5 cm in size and a few millimeters thick. Spot densities have reached the 10,000 spots/cm^2 range. Another way of making arrays of DNA is by means of high speed robotic printing or spotting onto glass slides [7]. This is a less expensive and more practical alternative to photolithography, since it uses simple capillary tips for the deposition of the reagents. The spot densities that can be achieved with this approach vary from 100 to 2500 spots/cm^2. Both of the previously mentioned techniques are primarily being used for sequencing and screening of oligonucleotides in studies involving genomics, drug discovery, and diagnostics. Since it is estimated that there are about 100,000 genes in the human genome and new ones are being identified every day, the practical potential of these high density techniques is tremendous especially in the area of drug discovery [8]. Roger Ekins [9] has described the fundamental principles involved in microspot microarray analysis. The primary advantage of microspot-based diagnostics over other formats such as those based upon 96-well microtiter plates is the positive correlation between increasing signal to noise ratios and diminishing diameters of microspots

* Corresponding author. Tel.: +1-530-752-7519; fax: +1-530-752-1537.

0003-2670/00/$ – see front matter
PII: S0003-2670(00)00776-5

down to a theoretical limit of about 3.5 μm. Fluorescence confocal microscopes or CCD cameras are most commonly used to detect microspots. A major advantage of miniaturized diagnostic arrays over standard methods such as those that use high performance liquid chromatography (HPLC) is their ability to conduct highly parallel simultaneous analysis. Chromatograph methods such as HPLC are normally performed in series. The parallel nature of microarrays also makes it possible to run many replicates of single datum points to increase precision.

The microarray-related works described in the literature have used a rectangular coordinate system to index individual microspots (Fig. 1A and B). In other words, each microspot has a corresponding unique (x, y) coordinate designation in a plane, usually a silicon chip or a glass slide. The use of a rectangular coordinate system is a natural choice especially for nucleotide synthesis and combinatorial chemistry applications that involve criss-crossing patterns of reagent deprotection and exposure to chemicals and light for the purpose of fabricating individual elements of a microarray. When the time comes to analyze the rectangular microarrays with CCD cameras or confocal microscopes, their rectangular arrangement also simplifies and facilitates their recognition and analysis by computer algorithms. Although rectangular arrangement of microarrays has proven to be adequate, such a coordinate system is not the only one by which microarrays could be arranged.

Despite the fact that discs have been used for the storage of analogue data (e.g. vinyl music records) and digital data (e.g. magnetic floppy discs and optical compact discs) for many years, the deposition of microspots in a circular fashion onto a disc (Fig. 1C) has not been explored. Utilizing these types of microarrays would involve the use of polar coordinates (r, θ), instead of the rectangular coordinates (x, y), to index each discrete microspot. There are several reasons why the use of disc-based microarrays for diagnostics purposes should be investigated:

1. The technology for handling and probing discs is very well established, robust, and low in cost. This observation is especially true for optical compact discs [10], on which data are stored in a spiral fashion (Fig. 1D). The cost of CD-based technology runs in the hundreds of dollars and compares very favorably to CCD cameras and lithographic equipment whose cost runs in the tens of thousands of dollars.
2. There is a lot of potential for overlapping/coordinating the activity of data analysis and data storage on a single disc medium. For example, a diagnostic microarray in circular or spiral form could be located on one side of a disc while the other side or other area of the same side could hold digital recordable media for the storage of analysis protocols or analysis results. This combination bodes well for potentially performing simultaneous, multianalyte, microarray-based analysis in the field utilizing an instrument similar in size to portable CD player. This instrument might perform the diagnostic analysis using the microarray on one side of the disc, utilizing stored protocols from and storing analysis results to the other side of the disc containing recordable media.
3. Disc supports are ideal for the collection of continuous flow samples deposited in the form of spiral microlines. Continuous flows of samples may come from a modern chromatographic instrument such as a microbore HPLC. Capillary deposition of the sample onto the disc would take place so that

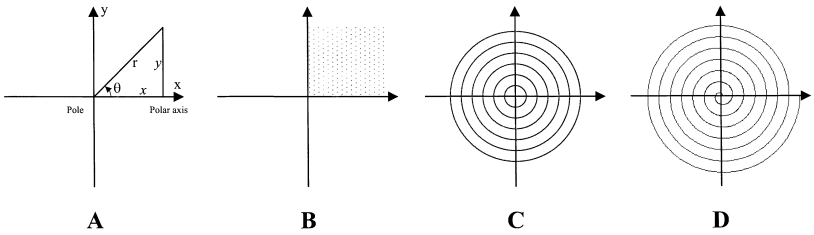

Fig. 1. Coordinate systems for microarrays. A: relationship between rectangular coordinates (x, y) and polar coordinates (r, θ). B: microarray in terms of rectangular coordinates (x, y). C: disc-based microarray in terms of polar coordinates (r, θ) where $r^2 = x^2 + y^2$ and $\theta = \tan^{-1}(y/x)$ if $x \neq 0$. D: CD digital data storage in terms of polar coordinates (r, θ) where $r = a\theta$ and a = constant (Archimedes' spiral).

the disc would function as a 'sample holder' ready to be probed by a method such as MALDI-MS.

To begin to explore the principle of disc-based diagnostic microarrays, we successfully combined low-density microarrays applied via piezoelectric inkjet applicator with circular indexing on a polycarbonate disc and used them to simultaneously run our competitive inhibition immunoassays for hydroxyatrazine, carbaryl, and molinate. Hydroxyatrazine is the product of hydrolysis of atrazine, a herbicide that is widely used in the world for the protection of crops such as corn. Carbaryl is a common pesticide used in the agriculture business. Molinate is a herbicide commonly applied to rice fields. Hydroxyatrazine, carbaryl, and molinate were selected as model compounds in our experiments because they represent potential contaminants in water whose simultaneous detection might be important for environmental studies and regulatory activities. The value of disc-based microarrays for quantitative analysis was demonstrated by generating a dose-response curve with the hydroxyatrazine immunoassay. Even though we demonstrated the principle of disc-based microarrays with competitive inhibition immunoassays, other types of previously published microarray-type of applications such as those involving oligonucleotides could also be implemented.

In addition to the previously mentioned experiments, we also confirmed that a CD surface could be a suitable sample holder for use in MALDI-MS analysis. In a simple experiment a circular polycarbonate fragment of a CD was coated with gold and used to detect proteins.

2. Experimental

2.1. Reagents

For the carbaryl immunoassay, antiserum Ab2114 and coating antigen (cAg) 5cona have been described [11]. Antiserum Ab4652 and cAg 7 that were used in the hydroxyatrazine immunoassay have also been described [12]. For the molinate immunoassay, Ab245 and cAg 7b-cona were described [13]. Goat antirabbit IgG was purchased from Sigma (St. Louis, MO) and labeled with Cy5 dye obtained from Amersham Life Science (Pittsburgh, PA) to make the fluorescent tracer as per the instruction insert from Amersham. The excitation maximum of Cy5 is at 649 nm while its emission maximum is at 670 nm. The versa-clean detergent was purchased from Fisher Scientific (Pittsburgh, PA). All salts were purchased from JT Baker (Phillipsburg, NJ), Fisher Scientific, or Aldrich Chemical (Milwaukee, WI). A Sybron/Barnstead Nanopure II water system set at 16.7 MΩ cm provided water for all aqueous solutions. The ethylene glycol was purchased from Sigma (St. Louis, MO). Dilution of immunoreagents for the purpose of conducting the incubations and in-between washes was carried out with 0.1 M phosphate buffered saline containing 0.05% Tween 20 (PBST), pH 7.5 (phosphate buffered saline: 8 g/l NaCl, 1.15 g/l Na_2HPO_4, 0.2 g/l KH_2PO_4, 0.2 g/l KCl). The standards of molinate, melamine, and hydroxyatrazine were obtained from DuPont (Wilmington, DE). Union Carbide (Danbury, CT) provided the carbaryl standard. The mixture of standard proteins used in the MALDI-MS demonstration was obtained from Hewlett–Packard (Palo Alto, CA).

2.2. Apparatus

The apparatus for depositing the immunoreagents onto the disc was improvised from a Stylus Color IIs brand, model p870a inkjet printer from Epson America (Torrance, CA) and a generic 5.25 in. floppy disk drive. A digital interface circuit was designed and constructed to operate the apparatus by means of a basic program running on a personal computer. Fig. 2 provides a schematic diagram of the whole system. Operation of the system was as follows: a disc stepper motor controlled by a personal computer rotationally positioned the disc solid support coated with immobilized immunoreagents. A printhead used for dispensing droplets of reagents onto the solid support surface was connected via a sliding mechanism to a stepper motor for positioning the printhead radially over the support surface. The printhead was comprised of three sets of arrays with 20 orifices each, one set for each 'ink'. Each array was connected to a separate syringe cylinder (not shown) holding a different reagent. The position of the printhead was controlled by the personal computer through the printer port for accurate alignment over the solid support surface so that the reagents could be applied to specific locations on the solid support surface according to a predefined pat-

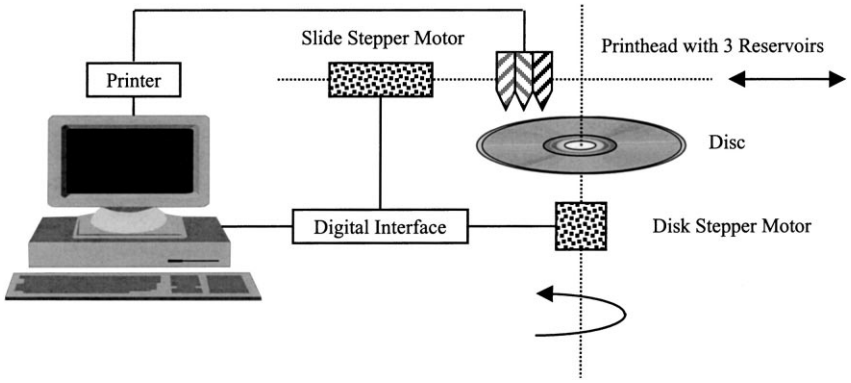

Fig. 2. Schematic diagram of the system for the deposition of droplets onto a disc.

tern. Fig. 3 is a picture of the actual apparatus that was deployed. The disc stepper motor was capable of rotating a 360° turn in 638 steps (minimum increments of θ approximately 0.56°) and the sliding mechanism was able to traverse the radius of the disc in 44 steps (44 different values of r). These parameters of the stepper motors made it possible to divide a standard 5.25 in. polycarbonate disc into 28,072 discrete areas. The fluorescent microspots on the discs were scanned with a Storm fluorescent scanner (Molecular Dynamics, Sunnyvale, CA) in the red fluorescence mode (excitation at 635 nm). The maximum resolution of the scanner was 50 μm. The scanner was controlled with a Macintosh Performa 6115CD personal computer running Molecular Dynamics' Scanner Control software version 4.1. Molecular Dynamics also provided the ImageQuant software version 1.2 that was used to quantify the signal levels from individual microspots.

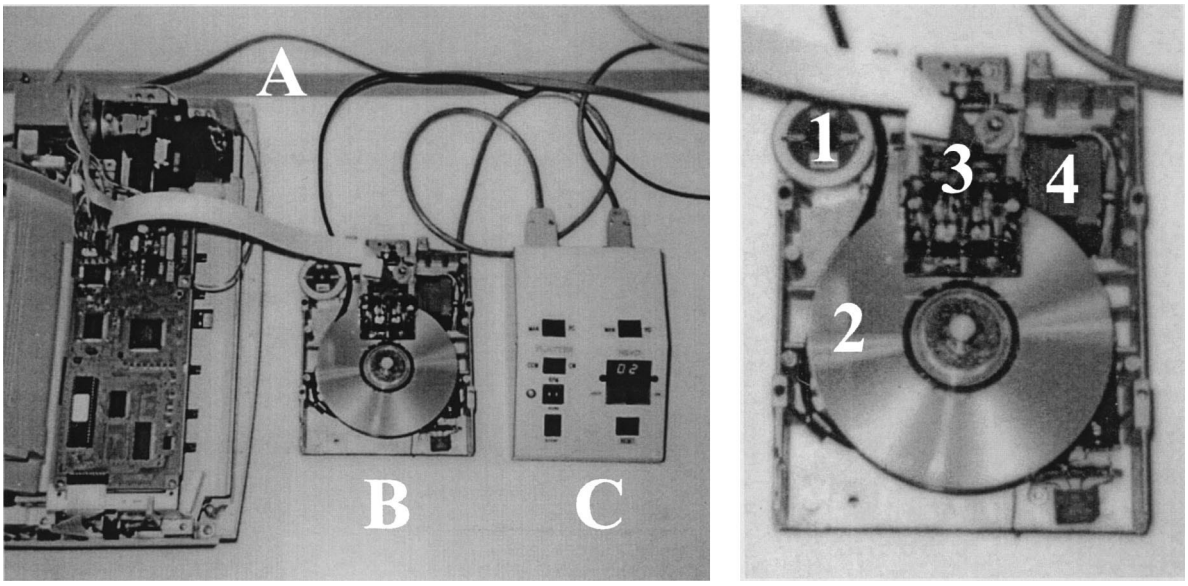

Fig. 3. Picture of system for the deposition of droplets into a disc utilizing off-the shelf materials. A: printer assembly, B: disc and piezoelectric printhead platform, C: computer interface, 1: stepper motor for rotating disc, 2: compact disc, 3: piezoelectric printhead with three reagent channels, 4: stepper motor for moving printhead sliding mechanism.

A Fisher brand micro-centrifuge, model 235B made by Fisher Scientific, was adapted for washing the discs. The discs themselves were obtained free of charge from various sources, which included America Online (Dulles, VA), and Microsoft Corp. (Bellevue, WA). The MALDI-MS system was a model g2025a 1d-tof made by Hewlett–Packard.

2.3. Procedure

The general procedure consisted of three steps. These were the immobilization step, the competitive inhibition step, and the development step with a fluorescent tracer (Fig. 4). Compact discs were gently washed with versa-clean detergent, rinsed with Nanopure water, and allowed to dry at room temperature. The application of approximately 1 μg/ml each of coating antigens 5cona, 7, and 7b-cona, in 50 mM carbonate buffer, pH 9.6 (30% ethylene glycol) was performed either manually with a 10 μl pipette tip or via the printhead. Appropriate sections of the printed areas were delineated with a black colored marker to serve as references and to help contain incubation solutions in subsequent steps of the assay. The coated discs were stored in petri dishes with water-saturated filter paper for 1 h at room temperature and then washed with PBST. Mixtures of 50% volume 100 μM analyte(s) plus 50% volume of 1/200 dilutions of rabbit antisera (polyclonal antibodies) Ab2114, Ab4652, and Ab245, specific for carbaryl, hydroxyatrazine, and molinate, respectively, were applied to the appropriate regions demarcated by the previously applied black-colored marks. All reagents were dissolved in PBST. After another incubation at 100% moisture for 1 h the disc was washed again with PBST. A solution of goat anti-rabbit IgG labeled with Cy5 fluorescent dye diluted 1/64 in PBST was placed on all of the reaction surfaces of the disc followed by another incubation at 100% moisture for 1 h. After a final wash with PBST the disc was scanned with a Storm fluorescence scanner interfaced with a personal computer running the Scanner Control software. The ImageQuant software was used to quantify the fluorescence signals from individual microspots.

For the MALDI-MS experiment, a compact disc fragment 9 mm in diameter was cut from a normal compact disc, its polycarbonate surface was covered with gold via vapor deposition, and placed on top of the original sample holder of the MALDI-MS instrument. A mixture of 7 μM Arginine-8-Vasopressin (1084.25 Da), 10 μM Angiotensin I (1281.49 Da), 10 μM Somatostatin (1637.90 Da), and 10 μM Chicken Atrial Natriuretic Peptide (3160.66 Da) in water was mixed 1:1 with sinapinic acid and was placed on the disc and dried under a vacuum. The prepared sample holder with the sample was then placed in the instrument and analyzed. The following instrument conditions were set: laser energy, 0.08 μJ; vacuum, 2.95e−006 Torr; ion optics 28.0/7.0 kV; detector, −4.75 kV; digitizer 1000 mV FS; polarity, positive; data interval, 5.0 ns; mass filter, 500 Da; mass range, 12,000 Da; and no filter. Four distinct peaks corresponding to the previously mentioned molecular weights were identified. This procedure was repeated using an original unmodified sample holder (coated with gold) with the same instrument settings except that the laser power was set to 0.05 μJ. The same four peaks were identified with this control experiment.

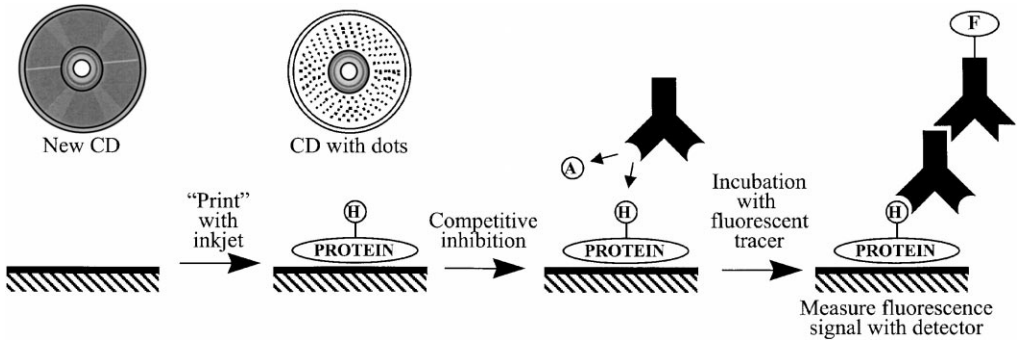

Fig. 4. Schematic diagram of the different steps of a competitive inhibition immunoassay in disc-based microarray form.

3. Results

We first conducted an experiment to demonstrate the principle of competitive inhibition diagnostic microarrays on compact disc by applying the array manually with a 10 μl pipette tip. The diameter of each dot in the array was approximately 800 μm. The disc was handled as described in the section on materials and methods. In the final step, the fluorescence signals emanating from the array were measured with a resolution of 50 μm. As can be seen from the scanned image of the microarray (Fig. 5), melamine at 100 μM did not appear to inhibit the immobilization of the first antibody to the surface of the disc while hydroxyatrazine gave maximum inhibition at this concentration. The average signal level at each concentration of analyte, as well as the corresponding standard deviation values, were computed and graphed (Fig. 6). The standard deviations of individual datum points were high because the corresponding dots were irregular in shape and size as a result of their manual application to the surface of the disc. Nevertheless, the levels of fluorescence were similar at low concentrations of melamine and hydroxyatrazine and very different at high concentrations of those chemicals.

In light of the successful demonstration of quantitative analysis with manually applied spots, the piezoelectric printhead was constructed (Fig. 3) and used to apply microspots to the disc to evaluate its performance. The graph in Fig. 7 is a composite dose-response curve of an hydroxyatrazine immunoassay run 4 times with 10 replicates per datum point. The linear range extended from 0.1 nM to 1 mM hydroxyatrazine, or approximately 7 orders of magnitude. These results confirmed that quantitative analysis is possible using microspots on a disc applied with a piezoelectric inkjet printhead. This same immunoassay run in 96-well microtiter plate format gave a linear response extending only 2 orders of magnitude from 1 nM to 0.1 μM hydroxyatrazine [12].

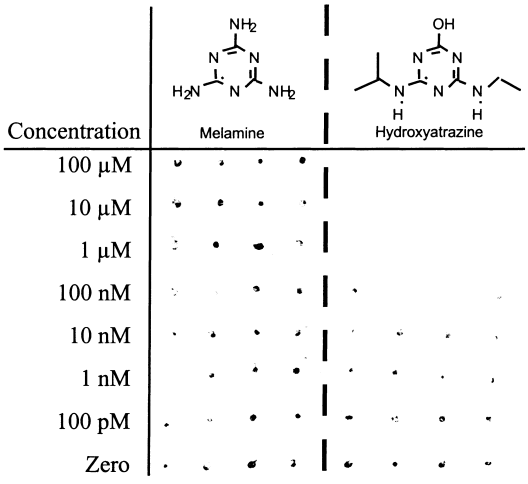

Fig. 5. Image of manually applied microspots. Competitive inhibition by hydroxyatrazine is evident. Melamine expresses no inhibition.

We also conducted an experiment to demonstrate that simultaneous analysis of multiple analytes is possible with a disc-based diagnostic microarrays. The coating antigens corresponding to immunoassays specific for three analytes were deposited with an Epson inkjet printhead. The dots were arranged in three concentric rings, one for each coating antigen. The innermost ring corresponded to the hydroxyatrazine immunoassay, the middle one to carbaryl, and the outer one to molinate. In Fig. 8, the image with zero on top corresponds to the situation in which none of the previously listed analytes were present in the competitive inhibition step of the assay. As a consequence, the primary antibodies in the incubation mixture corresponding to the three immunoassays were able to bind to their respective target coating antigens. This resulted in fluorescent signals from each ring sector. For the image labeled as HA, the incubation mixture at the competitive inhibition step contained 100 μM hydroxyatrazine. This is why the primary antibody specific for the hydroxyatrazine was not able to bind to the innermost ring of coating antigen, resulting in no fluorescent signal. In this same fashion, the images labeled as C and M corresponded to sectors in which 100 μM carbaryl and 100 μM molinate were present, respectively, causing the signals from the middle ring sector and the outermost ring sector to disappear. In the sectors corresponding to images labeled HA+C, HA+M, and C+M, the incubation mixtures contained 100 μM hydroxyatrazine plus 100 μM carbaryl, 100 μM hydroxyatrazine plus 100 μM molinate, and 100 μM carbaryl plus 100 μM molinate, respectively. In each one of these sectors, the analytes that were present in the mixture inhibited their specific antibodies resulting in the loss of fluorescent signal. The

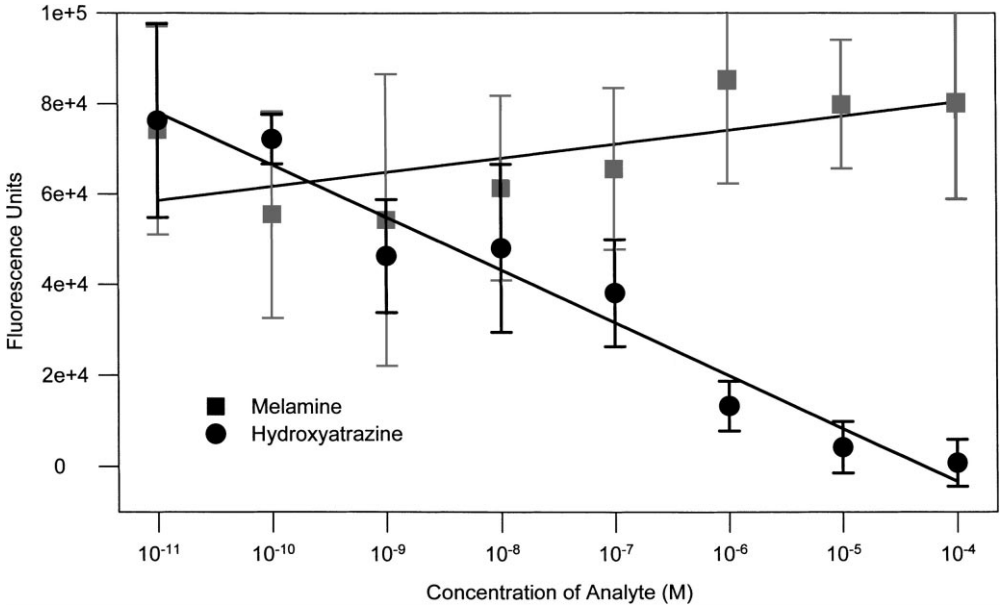

Fig. 6. Graph of data presented in Fig. 5.

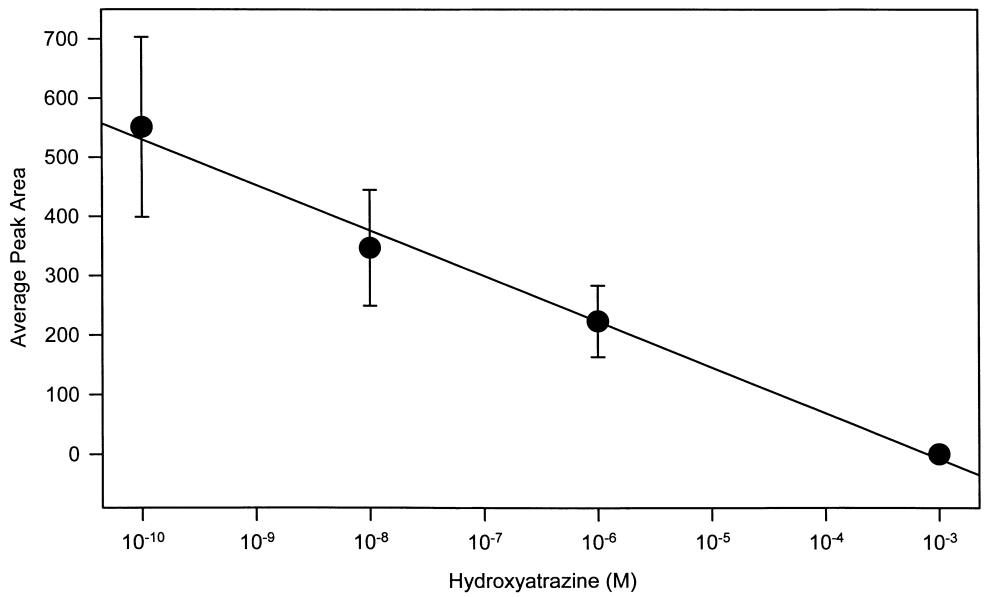

Fig. 7. Composite dose/response curve resulting from exposing dots (applied by inkjet apparatus) to different concentrations of hydroxyatrazine. The assay was run four times with 10 datum points per replicate.

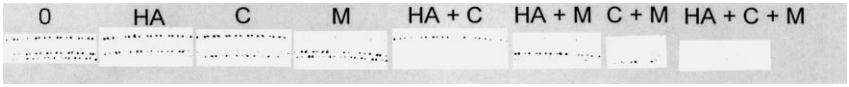

Fig. 8. Images demonstrating the simultaneous detection of molinate, carbaryl, and hydroxyatrazine. Dots were deposited with the piezoelectric inkjet apparatus in Fig. 3.

last image labeled HA+C+M belongs to the sector whose incubation mixture contained 100 μM each of hydroxyatrazine, carbaryl, and molinate. No visible fluorescent signals were detected here.

After having demonstrated the principle of quantitative and qualitative analysis utilizing disc-based microarrays, we briefly focused on the characteristics of individual microspots. One thousand individual microspots composed of the coating antigen for the hydroxyatrazine immunoassay were deposited on a disc and the immunoassay was carried out in the absence of any hydroxyatrazine. This resulted in maximal fluorescence signal from each microspot. The array was scanned and analyzed to determine areas of individual microspots and thus deduce their diameter distributions and average pixel intensity. The diameter frequency distribution is presented in Fig. 9. The diameters ranged from about 30 μm to approximately 130 μm, with 75 μm being the most common diameter.

4. Discussion

There are several ways of applying microspots to a surface. We used the piezoelectric inkjet approach. A good characteristic of this system is that once loaded into the inkjet reservoir, the sample can be applied a large number of times very quickly and efficiently. This is especially useful in applications such as the manufacturing of dipstick-type immunoassay devices where two or three reagents are applied in parallel lines. A drawback to using inkjet devices to apply microspots is that the number of different dots of different reagents is limited by the availability of inkjet reservoirs and the amount of time it takes to flush the system of one reagent and replacing it with another. Our system only had three reservoirs but a 10-reservoir printhead has been constructed [14]. In theory a large number of reagents in their respective reservoirs may be applied via a smaller number of output channels by the use of a system of lines and valves [15] but we do

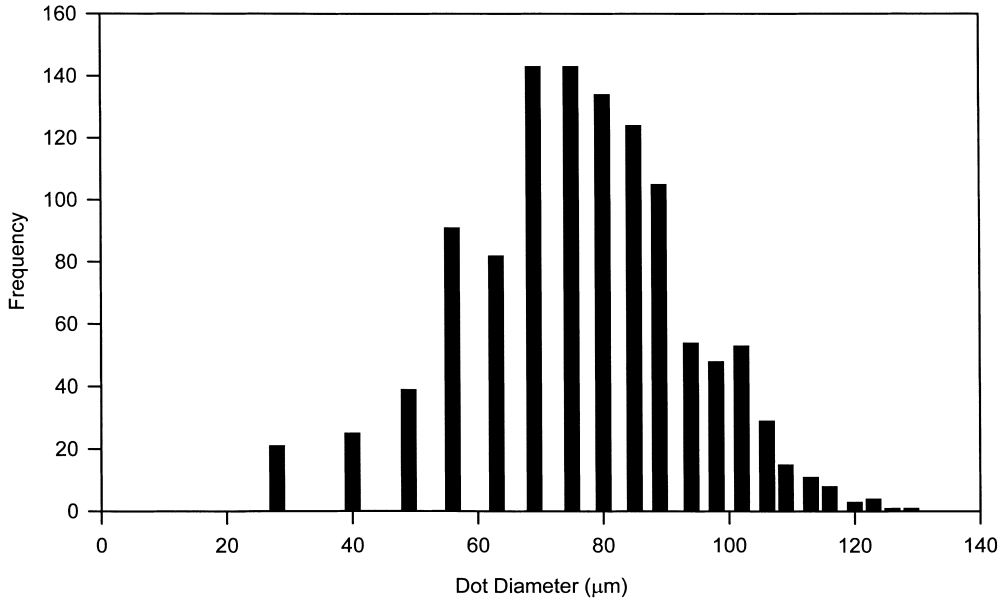

Fig. 9. Dot diameter histogram of 1000 dot replicates applied with inkjet technology.

not know how practical or reliable this system might be. A general drawback of the inkjet device that we tested is that it is susceptible to the formation of gas bubbles and to clogging that readily prevent the proper discharge of reagents from the inkjet orifice.

One method that is ideally suited for the application of a large number of different reagents is the capillary tip [7]. In contrast to an inkjet system, after the application of one reagent, the tip of a capillary device is very easy to wash during its preparation for use with another reagent. This washing procedure is amenable to automation and is practical and reliable. Several robotic devices for automatically applying dots of reagents from reservoirs such as 96-well or 386-well polystyrene plates have been described. Capillary, lithographic, as well as inkjet technology all should be applicable to applying samples to a CD surface.

Future work include the following:

1. Deploy a constant angular velocity (constant RPM) system with optical detection of a fixed reference point on the edge of the disc. A system with this combination of features will enable the automatic reorientation of a disc, after its removal from the spindle. Our current system cannot automatically orient the disc after its removal from the spindle.
2. Evaluation of a capillary-based microspot application system because it is more amenable to multiple sample application than inkjet technology.
3. Demonstrate the inherent flexibility of disc-based diagnostic microarrays by exploring other specific applications. The disc-based microarray system is ideal for the deposition of a spiral microline (Fig. 1D) from a flow sample source. For example, it may be possible to couple such a system to a microbore HPLC and use the disc as a continuous flow sample collector (Fig. 10). Placed in a MALDI-MS vacuum chamber, the rotating disc would serve as a platform for laser-induced desorption of the sample spiral on its surface. Indexing of the sample spiral would enable the user to know the exact retention time of every discrete point on the sample spiral. As described earlier, a fragment of a compact disc was coated with gold and used as a support for conducting MALDI-MS analysis of a mixture of several peptides. The results of the analysis were compared with those obtained with the normal gold-coated support that came with the instrument to yield very similar results (Fig. 11). By purchasing discs lacking the final plastic layer, one can have a gold surface that is compatible with MALDI-MS. In this application the compact disc can be used as a fraction collector for microbore HPLC or other chromatographic systems using the same device as has been described for the ELISA work. The effluent from the HPLC would be applied as discrete drops or continuous stream to locations indexed digitally. For sequencing, mass spectral analysis, or other analytical chemistry the location of fractions would be located digitally.

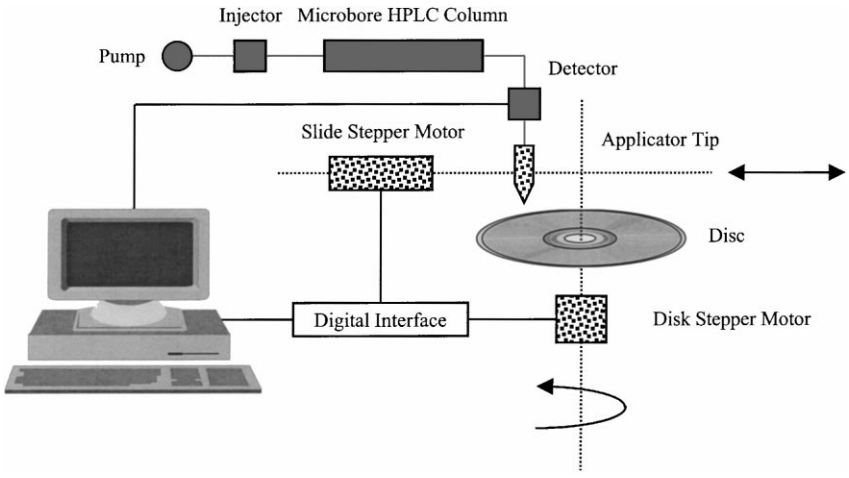

Fig. 10. Disc apparatus as a continuous flow sample collector for microbore HPLC.

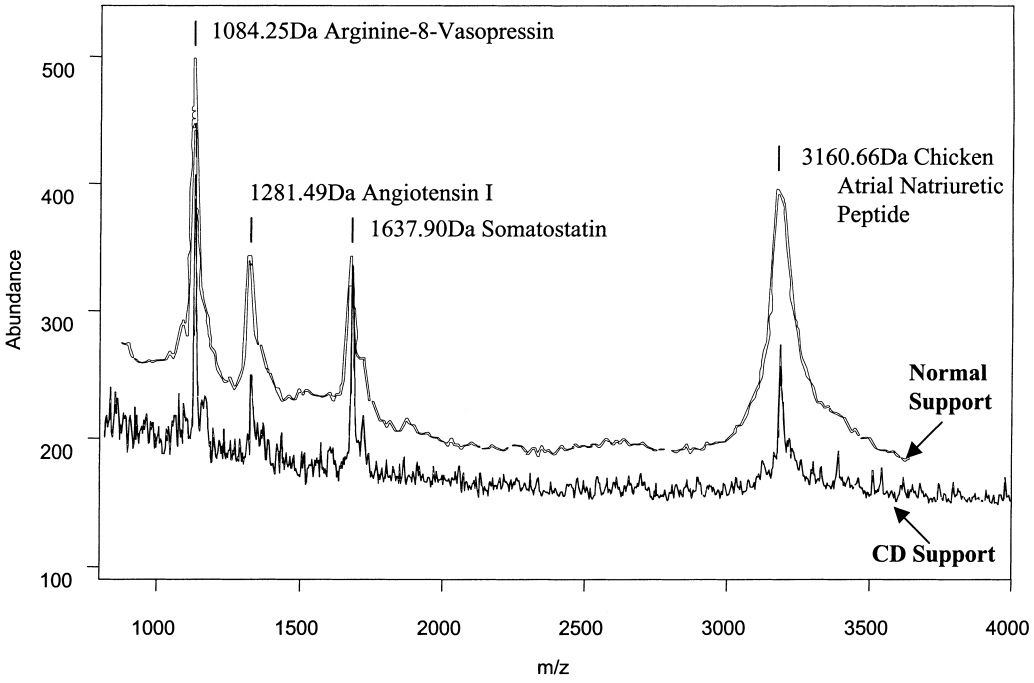

Fig. 11. Comparison of CD and normal supports for conducting MALDI-MS analysis of Arginine-8-Vasopressin, Angiotensin I, Somatostatin, and Chicken Atrial Natriuretic Peptide. Similar results were obtained for both supports. All conditions other than the support were identical between the two runs.

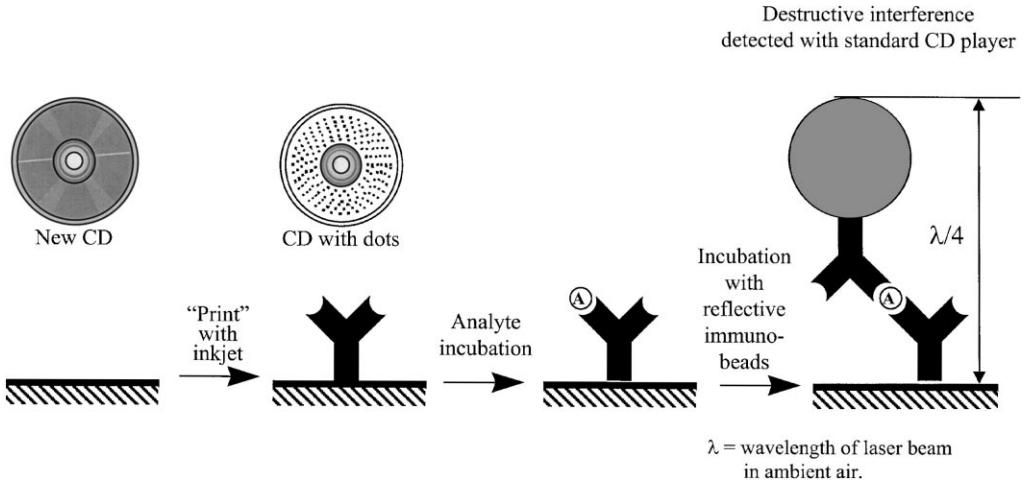

Fig. 12. Concept of reflective immunobead-based sandwich assay on CD. Light reflected from the reflective surface of the disc would be detected. However, light reflected from the reflective beads would cause destructive interference and would not be detected. The difference in height between the top of the bead and the reflective disc layer would be equal to one quarter the wavelength of the laser beam used (λ/4). This system would allow the use of standard CD ROM drives for analysis of the disc without any modifications.

4. The analysis of disc-based microarrays without the need of expensive visualization devices such as fluorescence scanners and microscopes would make disc-based microarrays very practical. Fig. 12 presents a concept that would enable the use of standard CD-ROM drives such as those commonly found in personal computers to analyze disc-based microarrays. Standard CD-ROMS use the principle of destructive interference to obtain the binary information from a CD (Sherman, 1988). The information on a CD is read by shining a laser beam onto the disc and detecting the strength of the reflection. The intensity of the reflected beam is dependent upon the presence of reflective pits and lands within the clear substrate of the disc (usually polycarbonate). The depth of the pit was chosen to be one quarter the wavelength (λ) of the laser beam in the transparent substrate (refractive index n=1.5). The reflected light from such a pit ends up 180° out of phase with the light reflected from the adjacent reflective surface causing destructive interference between the two reflections. If one were to run a reflective bead-based sandwich immunoassay on a CD with a single reflective layer and the distance between the top of the bead and the CD reflective surface were $\lambda/4$ it should be possible to see the same destructive interference when light is reflected from the reflective bead relative to the CD's reflective surface. For the purpose of surface immunoassay the value of $\lambda/4$ would depend on the refractive index of the laser in ambient air. The reflective beads might be gold particles that are commercially available. This format would enable the use of standard CD-ROM drives for the analysis of disc-based microarrays.

The conclusion from this work is that multianalyte competitive inhibition immunoassays can be carried out in circular miniaturized array format on discs. Piezoelectric inkjet technology provides one way to deposit microarrays onto a disc. The linear range of this application can span seven orders of magnitude making it potentially valuable not only for qualitative, but for quantitative analysis as well. The ready availability of equipment for reading discs and for writing to them makes this application of immunoassay even more valuable because in theory, a disc could be used not only to perform analysis, but also to store the resulting information.

Acknowledgements

This work was supported in part by the NIEHS Center (ES05707), the NIEHS Superfund Grant (ES04699), the US EPA (CR814709010), the US EPA Center for Ecological Health Research (CR819658), the NAPIAP (G59520062), and Tektronix Inc. (Beaverton, OR).

References

[1] N.L. Alpert, Clin. Chem. 15 (1969) 1198.
[2] L.J. Kricka, P. Wilding, in: G.J. Kost, J. Welsh, M. Kost (Eds.), Handbook of Clinical Automation, Robotics and Optimization, Wiley, New York, 1996, p. 45.
[3] A. Van den Berg, P. Bergveld, Micro Total Analysis Systems Proceedings of the μ TAS'94 Workshop held at MESA Research Institute, 21–22 November 1994, University of Twente, The Netherlands, Kluwer Academic Publishers, Dordecht, 1995.
[4] L.F. Rozsnyai, D.R. Benson, S.P.A. Fodor, P.G. Schultz, Angew. Chem. Int. Ed. 31 (1992) 759.
[5] C.P. Holmes, C.L. Adams, S.P.A. Fodor, P. Yu-Yang, Perspectives in medicinal chemistry, in: B. Testa, E. Kyburz, W. Fuhrer, R. Giger (Eds.), Helvetica Chim. Acta, Basel, Switzerland, 1993, p. 489.
[6] C.P. Holmes, S.P.A. Fodor, Innovation and perspectives in solid phase synthesis: peptides, proteins and nucleic acids, biological and biomedical applications, in: R. Epton (Ed.), Collected papers 3rd International Symposium, Mayflower Worldwide Ltd., Birmingham, UK, 1994, p. 221.
[7] M. Schena, D. Shalon, R.W. Davis, P.O. Brown, Science 270 (1995) 467.
[8] D.V. Patel, E.M. Gordon, Drug Discovery Today 1 (1996) 134.
[9] R.P. Ekins, F.W. Chu, Immunoanalysis of agrochemicals: emerging technologies, in: J.O. Nelson, A.E. Karu, R.B. Wong (Eds.), ACS Symposium Series 586, American Chemical Society, Washington, DC, 1995, p. 153.
[10] J.N. Nadler, R.J. Wiesenberg, in: C. Sherman (Ed.), The CD ROM Handbook, McGraw-Hill, New York, 1988, p. 79.
[11] M.P. Marco, S.J. Gee, H.M. Cheng, Z.Y. Liang, B.D. Hammock, J. Agric. Food Chem. 41 (1993) 423.
[12] H. Kido, M.H. Goodrow, V. Griffeth, A.D. Lucas, S.J. Gee, B.D. Hammock, J. Agric. Food Chem. 45 (1997) 414.
[13] S.J. Gee, T. Miyamoto, M.H. Goodrow, D. Buster, B.D. Hammock, J. Agric. Food Chem. 36 (1988) 836.
[14] D. Wallace, H.-J. Trost, U. Eichenlaub, in: W. Ehrfeld, I.H. Renard, R.S. Wegeng, Proceedings of the 2nd International Conference on Microreaction Technology, New Orleans, 9–12 March 1998, The American Institute of Chemical Engineers, New York, 1998, p. 129.
[15] D.J. Hayes, D.B. Wallace, C.J. Frederickson, Method and Apparatus for Making Miniaturized Diagnostic Arrays, United States Patent #5,658,802, issued 19 August 1997.