FAST™ Slides: A Novel Surface for Microarrays

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ABSTRACT

We have evaluated FAST slides, a glass slide with a microporous polymeric surface that is a suitable substrate for microarray technology. The surface is a nitrocellulose-based polymer that binds DNA and proteins in a noncovalent but irreversible manner. FAST slides are compatible with robotic systems currently used to create microarrays and can easily accommodate volumes of 0.03–2 nL/spot. Our data indicate that FAST slides have a much higher binding capacity for DNA and better spot-to-spot consistency than traditional poly-lysine-coated slides. In addition, FAST slides are well suited for fluorescent detection because of their relatively low light scatter and efficient retention of arrayed DNA. These properties translate into fluorescent sensitivity comparable to modified glass surfaces. FAST slides are also ideal for arraying proteins, making them the only substrate of their kind currently available for microarray applications.

INTRODUCTION

DNA microarrays are powerful tools for high-throughput gene expression analysis. With this technology, the expression patterns of thousands of genes can be examined simultaneously (7). To accomplish this, PCR libraries are robotically arrayed onto a modified glass slide surface and then probed. Most published procedures for creating arrays use poly-lysine-coated glass slides as the array surface (3). However, these slides are plagued by inconsistencies that often result in inconclusive data interpretation (1). Technical developments that offer increased surface consistency will undoubtedly increase the power of this technology. In this study, we explore the utility of FAST slides in microarray applications.

The surface of FAST slides is a proprietary nitrocellulose-based polymer. Nitrocellulose is a polymer that binds nucleic acids and proteins in a noncovalent but essentially irreversible manner and has been used in traditional blotting and binding applications for over 60 years (5,6,8). This microporous polymeric surface is ideally suited for microarrays and offers an alternative to poly-lysine slides that is easier to use and provides more consistent binding properties. In addition, problems such as uneven surface coating are avoided. Unlike other surfaces, FAST slides require minimal post-array processing. Irreversible binding of DNA occurs through a simple dehydration step involving no chemical modification.

Here, we report characteristics of FAST slides that are relevant to microarray technology. These include DNA binding capacity, behavior in hybridization reactions, optical properties relevant for fluorescence-based studies and the ability to array and detect proteins for use in proteomic applications.

MATERIALS AND METHODS

DNA and Protein Arraying

All arrays were spotted with a BioGrid benchtop robot (BioRobotics, Cambridge, UK). Biotinylated DNA samples for direct detection were serially diluted in 3× standard saline citrate (SSC) before spotting. DNA to be arrayed on FAST slides for hybridization experiments was resuspended in a chemical denaturation solution (3 M NaCl, 0.8 M NaOH). Arrayed slides that were not hybridized immediately were rinsed in a neutralization solution (0.5 M Tris-HCl, pH 7.0, 1.5 M NaCl) for 5 min. Purified protein samples were diluted in 1× PBS. Samples were spotted onto slides with a 0.2-mm diameter pin-tool. Resulting spots were between 200 and 250 µm in diameter.

Preparation and Post-Processing of Slides

Poly-lysine-coated glass slides were prepared as previously described (7). Briefly, glass microscope slides (Sigma, St. Louis, MO, USA) were cleaned in a 2-M sodium hydroxide/70% ethanol solution for 2 h. After being washed in distilled water, the slides were soaked in a 0.01% poly-lysine solution (Sigma) for 1 h. Slides were washed again for 1 h and dried for 5 min at 70°C in a vacuum oven.

After arraying, poly-lysine slides were rehydrated for 30 s in a humidified chamber and then snap-dried for 3 s on a 100°C hot plate. The DNA was cross-linked to the surface by exposure to 60 mJ UV light in a Stratalinker® (Stratagene, La Jolla, CA, USA). Further blocking of the poly-lysine slides used for the DNA binding capacity experiments was not necessary because these slides were not hybridized with probe DNA. The glass slides used in hybridization experiments were blocked in methyl pyrrilidinone containing 140 mM succinic anhydride and 20 mM boric acid for 15 min. After being incubated in a 95°C water bath for 2 min and then in 95% ethanol for 1 min, slides were dried with compressed nitrogen.

FAST slides were developed by Grace BioLabs (Bend, OR, USA) and Schleicher & Schuell (Keene, NH, USA). After arraying the FAST slides with DNA or protein, they were baked at 80°C for 30 min.

Chemiluminescent Detection of Nucleic Acids

pUC19 plasmid DNA was labeled with psoralen-biotin (Schleicher & Schuell) according to the manufacturer's instructions. Dilutions of biotinylated DNA were spotted (four replicates per row) onto FAST and poly-lysine slides. The highest concentration of DNA arrayed was 300 ng/µL, followed by 14 additional 1:2 dilutions. After processing, slides were incubated in a blocking solution containing 3% casein, 0.1% SDS and 1× TBS for 1 h. A streptavidin-HRP conjugate (Life Technologies, Rockville, MD, USA) was added directly to the blocking solution at a 1:1000 dilution, and slides were incubated for an additional hour. After a series of washes (0.1% SDS/1× TBS), chemiluminescent detection was performed using an ECL® kit (Amersham Pharmacia, Uppsala, Sweden) according to the manufacturer's instructions, and slides were exposed to film.

Light-Scatter Measurements

Light scatter from surfaces was measured with a modified inverted fluorescent microscope. Samples were illuminated with light from a 10-W tungsten halogen lamp passed through either a 488-nm DF22 (fluorescein) or 645-nm DF 70 (Cy5) excitation filter. Scattered light was collected through either a 520-nm DF30 (fluorescein) or 660-nm DF40 (Cy5) emission filter. The light intensity was measured by a digital voltmeter. All optical filters were purchased from Omega Optical (Brattleboro, VT, USA).

Hybridization of Fluorescently Labeled Probes

Cy™5-dCTP or Cy3-dCTP (Amersham Pharmacia Biotech) were incorporated into a pUC19 116-bp fragment using a standard PCR kit (PE Biosystems, Foster City, CA, USA). Unlabeled dCTP to Cy-labeled dCTP ratio was 1:1 in the reactions.

pUC19 plasmid DNA was spotted onto FAST slides in half-log dilutions, five replicates per concentration. The concentrations were 300, 95, 30, 9.5, 3, 0.95 and 0.3 ng/µL. After processing, slides were incubated in 3 mL prehybridization solution (5× SSC, 5× Denhardt's solution, 0.8% SDS and 100 ng/µL salmon sperm DNA) for 1 h at 42°C in a heat-sealed hybridization bag. The Cy5-labeled pUC probe was added directly to the prehybridization solution at a final concentration of 5 ng/mL, and the slides were incubated at 42°C overnight. Slides were then washed for 20 min each in 2× SSC/0.1% SDS at room temperature and in 0.1× SSC/0.1% SDS for 20 min at 65°C. After being dried in a 70°C oven, the slides were visualized in a ScanArray® 4000 fluorescent imager (GSI Lumonics, Watertown, MA, USA). For the FAST slide microarray imaging, the fluorescent imager was set at 92% laser power and 47% photomultiplier tube voltage (PMT). For the glass slide imaging, settings were 80% laser power and 80% PMT.

Chemiluminescent Detection of Proteins

Purified creatine kinase (Roche Molecular Biochemicals, Indianapolis, IN, USA) was arrayed onto FAST slides. The highest concentration spotted was 8 µg/mL followed by fourteen 1:2 dilutions (three replicates per row). After spotting, slides were blocked in 5% nonfat dry milk/0.1% Tween® 20/1× PBS and then incubated for 1 h with purified AH6 anti-creatine kinase monoclonal antibody diluted 1:1000 in 5% milk/1× PBS. After 4 × 15 min washes in 1× PBS/0.2% Tween

20, slides were incubated with an anti-mouse-HRP antibody (Amersham Pharmacia Biotech) diluted 1:2500 in 5% milk/1× PBS for 1 h. Slides were washed again (4 × 15 min, 1× PBS/0.2% Tween 20), and bound antibody was visualized by ECL.

RESULTS AND DISCUSSION

Quantitation of DNA Binding on Slides

Robotically spotted DNA must interact efficiently with the surface onto which it is arrayed. To examine the interaction of DNA with the FAST slide surface and with poly-lysine-coated glass slides, we arrayed serial diutions of biotinylated DNA. The arrayed DNA was visualized by chemiluminescent detection of streptavidin-HRP conjugate and exposure to X-ray film as described in Materials and Methods (Figure 1A). The signal was quantified by digitally scanning the exposed film (Figure 1B). Because a secondary conjugate molecule was used, this experiment measured arrayed DNA available for interaction (i.e., hybridization), not just bound DNA.

For any given concentration of DNA in the source plate, a more intense signal was observed from the FAST slide. At high concentrations (Figure 1A, rows 1–3), the increased signal indicated that the FAST surface had a much higher binding capacity, with more DNA available for hybridization (Figure 1B). At low concentrations (Figure 1A, rows 4–6), this translated into a sensitivity that was at least tenfold better than on poly-lysine (Figure 1B), which is not surprising since nitrocellulose membranes typically have an upper limit for DNA binding capacity of greater than 75 μg/cm^2 (data not shown). Because the binding of probe DNA to an array is a bimolecular reaction with an equilibrium that depends on the concentration of both reactants, increased availability of capture sequence translates into a better signal at lower probe concentrations.

In addition to higher binding capacity, DNA binding was more consistent from spot-to-spot on FAST slides. The coefficient of variation (CV) among spots from the same source plate concentration was substantially lower for FAST slides compared to poly-lysine slides (Figure 1C). Minimizing variability of spotting is essential in microarray applications, especially when comparing gene-to-gene expression levels. Large inherent variability created by the surface can add to the challenge of interpreting microarray data.

Optical Properties of FAST Slides

Microarrays are commonly hybridized with fluorescently labeled probes. With proper instrumentation, fluorescent detection can provide comparable sensitivity and a greater dynamic range than other detection methods such as the use of radioactivity or chemiluminescence. Furthermore, multiplexing, the use of two or more probe sets simultaneously, is easily accomplished by using probes labeled with two different fluorophores.

To determine the compatibility of FAST slides with light-based detection systems, we measured the intensity of scattered light that occurred from FAST slides when they were illuminated at two different wavelengths. Figure 2 shows the intensity of light scattered by traditional nylon and nitrocellulose membranes as well as a glass surface and FAST slides.

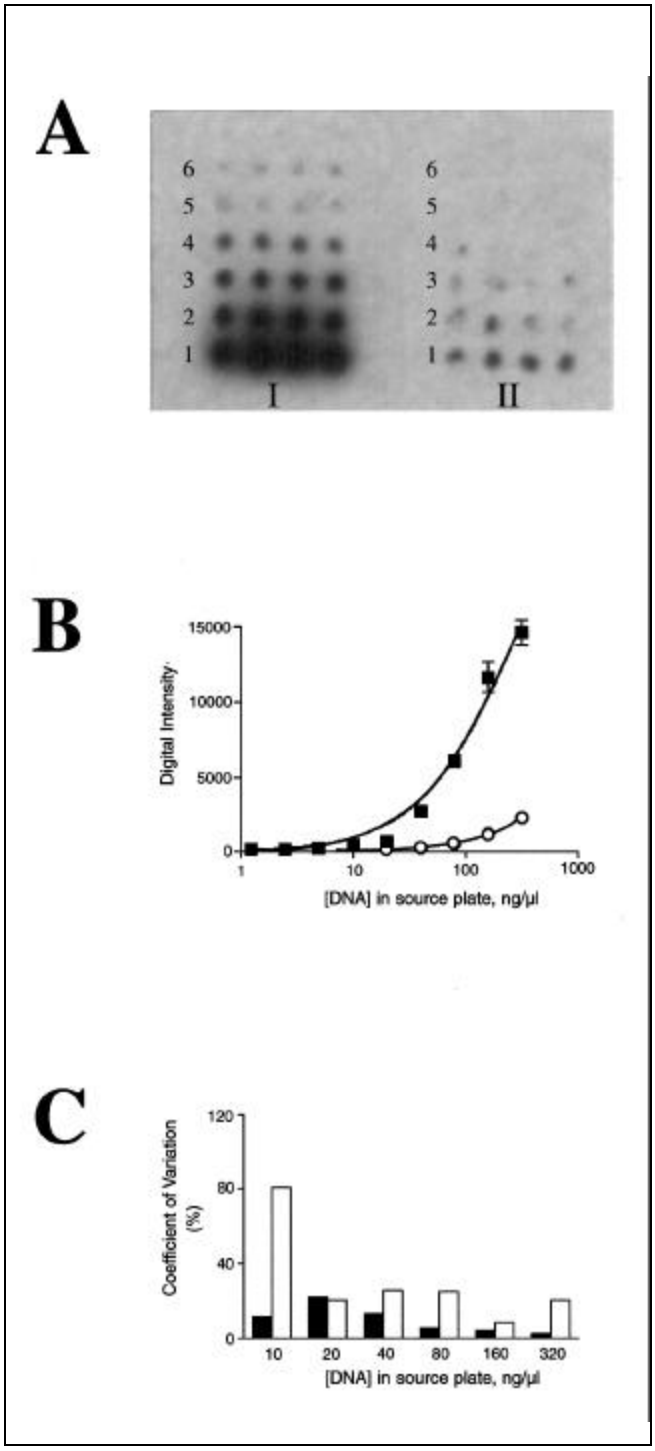

Figure 1. Chemiluminescent detection of biotinylated DNA on poly-lysine- and polymer-coated slides. (A) Quadruplicate columns of 1:2 dilutions (starting at 320 ng/μL) of psoralen-biotin-labeled DNA were spotted with a robot arrayer and immobilized onto slides (I: FAST slides; II: poly-lysine slides) and detected with the addition of a streptavidin-HRP conjugate and enhanced chemiluminescence. For the above experiment, the most concentrated DNA solution arrayed was 300 ng/μL and the film exposure time was 10 min. The positive signal was quantitated digitally using a flatbed scanner. Data are presented as background subtracted from a negative control spot containing no sample. (B) Shown here is the relationship between concentration of DNA arrayed and digital intensity, with the data from a single experiment performed in quadruplicate and is representative of three independent experiments. The error bars represent SEM. ■, polymer-coated slide; O, poly-lysine coated slide. (C) CV of the data points shown in (B) for the DNA concentrations that were detectable on both types of surface. Shaded bars, polymer-coated slide; open bars, poly-lysine-coated slides.

FAST slides had lower light-scatter intensity than other microporous surfaces at each emission wavelength examined (Figure 2). Note that nitrocellulose membranes in general scattered less light than nylon, even at comparable porosity. This difference is likely due to the nature of the two different polymers. FAST slides, which are approximately 80% thinner than traditional nitrocellulose membranes, demonstrated slightly better light-scatter properties than the nitrocellulose membranes.

FAST slides did scatter more light than clear glass slides. This was not an unexpected result, considering that the FAST slide surface is white. Although increased scatter contributes to increased noise in an optical measurement, the benefits of using a membrane-based slide can offset this effect. As mentioned above, the higher binding capacity of FAST slides results in better retention of arrayed DNA than on glass, increasing the amount of DNA available for hybridization and generation of signal. Thus, the net signal-to-noise ratio may not be adversely affected by increased light scatter.

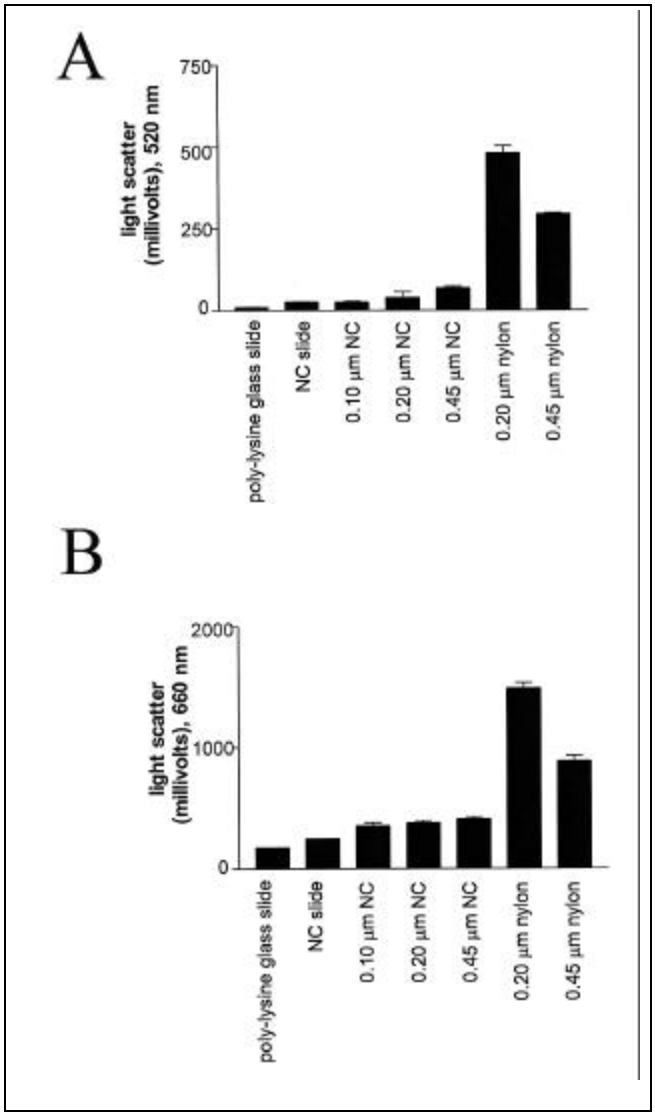

Figure 2. Light scatter from various surfaces at 488/520 nm and 645/660 nm excitation/emission wavelengths. Using a fluorescent microscope linked to a voltmeter, the light scatter reflected from various surfaces was measured using two different emission filters. Data shown are from an experiment performed in triplicate; error bars represent SEM.

Hybridization of Fluorescent Probes to Arrayed FAST Slides

The ability of DNA arrayed on FAST slides to hybridize with fluor-labeled probe DNA was also assessed. Serial dilutions of DNA were spotted onto FAST and poly-lysine-coated slides. The slides were incubated with a complementary Cy3- or Cy5-labeled DNA probe. Figure 3 demonstrates that the two surfaces have similar sensitivities for both Cy3 and Cy5. In the Cy5 experiment, a quantifiable signal was obtained from DNA arrayed at 3.0 ng/µL. For Cy3, sensitivity was reduced on both glass and FAST slides, with a threshold at 9.5 ng/µL.

The imaging of FAST slides results in a higher background signal because of the increased light scatter of the membrane surface. However, the background-subtracted signal on FAST slides was actually significantly greater than on glass for most of the DNA concentrations arrayed. For example, the average spot intensity (background subtracted) for the highest dilution on FAST slides was 45 000 U (Figure 3A, top row) compared to an average of 12 000 U on glass (Figure 3B, top row). These data demonstrate that the increase in bound DNA on FAST slides (Figure 1) leads to increased capture of probe DNA during hybridization. Although FAST slides have a

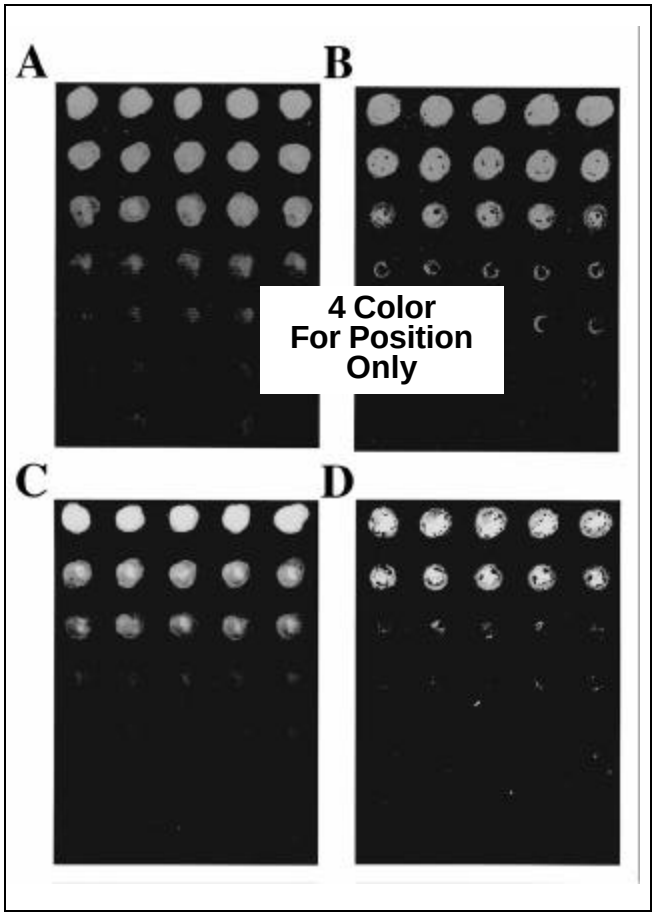

Figure 3. Hybridization of fluorescently labeled PCR probe to DNA arrayed on FAST slide. Half-log dilutions of pUC DNA (2.7 kb) were arrayed onto FAST (A and C) or poly-lysine-coated (B and D) slides. Each row contains five replicates of each concentration, which were 300, 95, 30, 9.5, 3, 0.95 and 0.3 ng/µL from top to bottom. The slide was hybridized with a Cy5-dCTP (A and B) or Cy3-dCTP (C and D) incorporated 116-bp pUC PCR fragment. Spots are approximately 200 µm in diameter and are 0.6 mm apart (from center to center). After washing, the slide was visualized in a fluorescent imager.

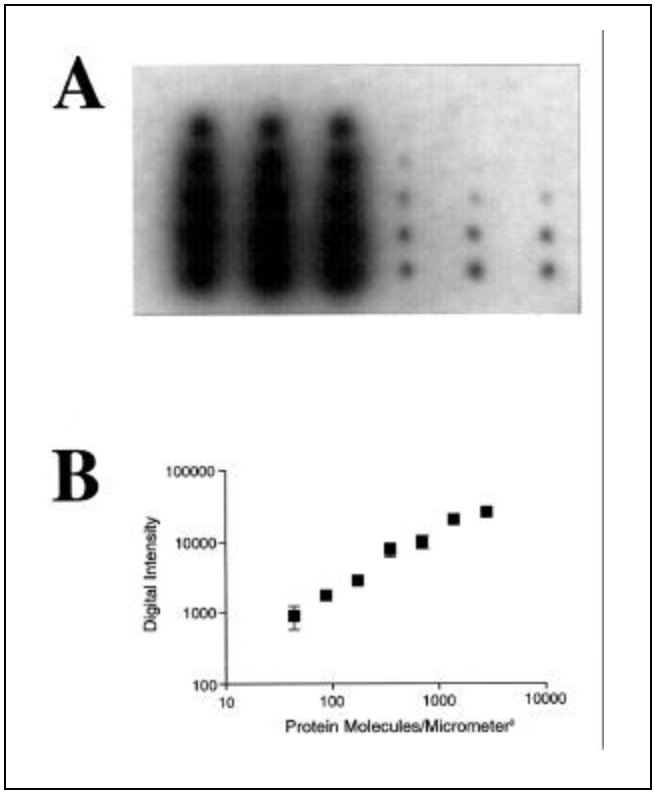

Figure 4. Chemiluminescent detection of purified protein on polymer-coated slides. (A) Triplicate columns of 1:2 dilutions (starting at 8 mg/mL) of purified creatine kinase were spotted with a robot arrayer and immobilized. After incubation with an anti-creatine-kinase antibody followed by a secondary HRP antibody, the proteins were detected by enhanced chemiluminescence. The most concentrated protein solution shown is 8 µg/mL and the film exposure time was 5 min. (B) The relationship between protein concentration and signal from digitally scanning the film is shown in (A). Data are presented as background subtracted from a negative control spot containing no sample and are representative of three independent experiments; error bars represent SEM

higher background than poly-lysine slides even before hybridization occurs, this background does not increase significantly after hybridization (data not shown). Note that the fluorescent measurements were taken at different imager settings for each of the two surfaces (see Materials and Methods). Optical imager settings were determined to minimize background while maintaining dynamic range; the white surface of the FAST slides makes this difference necessary.

The data in Figure 3 also provide evidence that the unique membrane surface of FAST slides leads to a more uniform array. It is apparent that the pattern of hybridized probe intensity within each spot of the glass arrays (Figure 3, B and D) is irregular and asymmetrical compared with the FAST spots (Figure 3, A and C). Quantitation of pixel intensity CV within each spot was 35%–40% for the coated glass arrays versus only 20% for the FAST arrays, confirming better spot morphology on FAST slides.

Protein-Based Applications

Proteomics, the study of the protein products of a genome, is an emerging field that aims to bridge the gap between genome sequence and cellular behavior (2). Protein microarrays may ultimately provide a tool for antibody screening, analyzing gene expression patterns and measuring low-affinity protein-protein interactions simultaneously (4). We examined FAST slides as a potential medium for the array and detection of proteins. FAST slides were arrayed with decreasing concentrations of a purified protein, creatine kinase. The array was probed with an anti-creatine kinase antibody and visualized via chemiluminescence as described in Materials and Methods (Figure 4A). Quantitation of the image by digital scanning is shown in Figure 4B. Using this method, a dynamic range of 3 logs was obtained, with a lower level of sensitivity at approximately 30–40 molecules/μm^2.

CONCLUSION

FAST slides are suitable substrates for a wide range of applications in high-throughput biology. They demonstrate better array consistency and higher binding capacity than traditional modified glass slides. The relatively thin surface coating creates a three-dimensional structure to absorb and bind arrayed DNA. FAST slides provide the researcher with an easy-to-use slide platform; they are ready for use right out of the box and require minimal post-array processing, (80°C, 30 min). The ability of the surface polymer to retain DNA and protein in a near quantitative manner allows greater flexibility than other surfaces for microarrays and can provide a consistent platform for the eventual progression of microarrays into diagnostics.

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