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Novel surface and multicolor charge coupled device-based fluorescent imaging system for DNA microarrays

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Abstract. We report a novel support, concomitant attachment chemistry, and a fluorescent imaging system for DNA microarrays. The support consists of soda lime glass coated with a layer of chromium, which eliminates any autofluorescence from the underlying glass substrate and reduces nonspecific probe binding. Attachment of DNA fragments exceeding 300 nucleotides in length is achieved without chemical modifications of either the chrome surface or the DNA itself. The charge coupled device (CCD)-based imaging system employs a 175 W xenon arc lamp as the light source, allowing the use of many different fluorophors. A 14 mm$\times$9 mm sample area is imaged with a single exposure, which takes between 5 and 20 s for each color plane in typical genomic comparative genomic hybridization type assays. The spatial resolution is limited only by the pixel size of the CCD chip (9 μm). The oblique illumination geometry combined with effective background reduction afforded by the chromium surface enables the system to achieve a detection limit of $<5\times10^7$ fluorophors/cm^2 with 10 s integration. In a model system with arrayed lambda DNA targets a dose response was observed over four orders of magnitude in response to hybridizations with increasing amounts of the fluorescent labeled lambda probe.
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1 Introduction

DNA microarrays consist of arrays of DNA sequences of varying length immobilized on a solid support. They have emerged as a powerful new tool for genomic analysis and gene expression profiling, with applications in DNA sequencing, gene discovery, drug discovery, and disease diagnosis.[1] For analysis the test DNA to be analyzed (referred to as the probe herein) is typically labeled with a fluorescent dye and then hybridized to the microarray. The extent of hybridization is then evaluated by a fluorescent imaging system with appropriate software.

The type of support and the imaging system used have a significant impact on the sensitivity, speed, and reliability of the assay. Glass slides have been most widely used in combination with scanning-based epi-fluorescence systems[2,3] that employ lasers as the excitation light source. A large numerical aperture (NA) objective is usually used to focus the excitation beam into a very small spot ($\sim$3–20 μm) and to collect the fluorescent emission over a large solid angle.[4] Coupled with a photomultiplier detection tube, these systems offer high resolution and sensitivity. The standard confocal design offers the capability of reducing substrate background by limiting the depth of focus and rejecting out-of-focus fluorescence emission.[5] Since only an area of about 9–400 μm^2 of the chip surface is illuminated by the focused laser beam at any given time, a mechanism which facilitates the relative movements of the focal point on the chip surface is required in order to scan the whole microarray. Consequently, data acquisition over an area of 1 cm^2 may take several minutes.[3] Another undesirable feature of laser-based scanning systems is the limitation they impose on the choice of fluorescent dyes.

Recently alternative solutions for imaging microarrays have emerged that employ charge coupled device (CCD) detectors and broad-spectrum light sources. CCDs are detectors in which a large number of pixels are arranged in a planar array, with each pixel functioning as an individual light intensity detector. With appropriate imaging optics, they can be used as a camera. The area that can be imaged with a single exposure depends on the resolution requirement, CCD chip dimensions and pixel size, and the magnifying power of the optics. In addition, they require a powerful light source that can illuminate the whole sample area with sufficient irridiance. Currently, the practical limitation on the number of pixels of a single CCD chip appears to be about 6 million (such as the Kodak KAF-6303 chip), allowing 6 cm^2 sample area to be imaged with 10 μm resolution. Chips with more than 16 million pixels are commercially available but are expensive.

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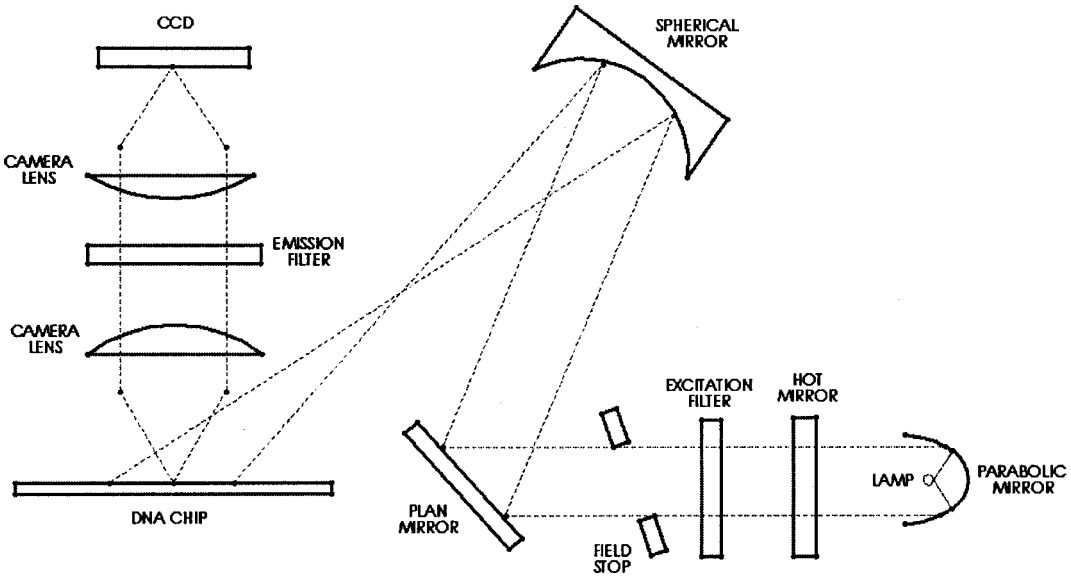

Fig. 1 Optical layout of the imaging system.

CCD-based imaging systems are generally associated with simplicity in design and rapidity in data acquisition. On the downside, imaging of a large area with sufficient image quality does not allow the rejection of out-of-focus fluorescence, meaning that autofluorescence emitting from the glass substrate can significantly limit the detection sensitivity of these systems.

Here we report a novel substrate for support of DNA arrays and a CCD-based multicolor fluorescent imaging system. Combined they provide a detection limit comparable to that of confocal scanning systems without forfeiting the speed and simplicity of CCD-based imaging.

2 Experiment

2.1 Imaging System

Figure 1 schematically depicts the design of the imaging system. The excitation light beam from a xenon arc lamp passes through an interference hot mirror (A200HMP, ILC) to block infrared frequencies and reduce heating on subsequent components. The beam then passes through a six-position filter wheel (AB-301, CVI Laser Corporation, NM) equipped with 25 mm diam single band pass interference excitation filters centered at 405, 490, and 570 nm, each with a bandwidth of 20 nm (61405, 61490, and 61570, Chroma Technology, VT). A plane mirror with a diameter of 50.8 mm (033-0250, OptoSigma Corporation, CA) is positioned to redirect the excitation light with incidence angle of 32.5° to a spherical mirror with a diameter of 50.8 mm and a radius of curvature of 250 mm (035-0350, OptoSigma). This concave mirror images an optional field stop onto the microarray surface, which allows adjustment of the size of the illuminated area by adjusting the field stop. The incident angle of the excitation light beam with regard to the chip surface is 45°, resulting in reflection of the excitation light without it entering the collection optics. The collection optical train consists of a pair of 50 mm/f1.4 camera lenses (50/1.4D AF Nikkor, Nikon, Japan) with a 50 mm diam triple band emission filter (61002m, Chroma Technology) mounted in between. The two lenses are used in a head-to-head configuration that was described by Wittrup et al.[6]

For the work described herein a CCD camera with a readout rate of 1.25 MHz and digital resolution of 12 bits (AP2, Apogee) served as the image detector. The readout noise was about 12 electrons. This camera is equipped with a 1536 $\times$1024 pixel array CCD chip, whereby each pixel measures 9 μm$\times$9 μm. The camera is thermal electrically cooled to -10 °C. At this temperature, the dark current was less than one electron per pixel per second.

For imaging, the DNA chip is placed on a precision mount with an *xy*-position locking mechanism. The tips of four pin stops define a reference plane, coinciding with the focal plane of the collection lens. The *z* position of the tips of the pins as carefully adjusted and then fixed. Initial focusing also involved fine *z*-adjustment of the collection lens while imaging was acquired and refreshed continuously. Once the best focus was achieved the lens position was locked. During routine use, the spring loaded mount pushes the chip surface against these stops, achieving effective focusing through positioning within a $\pm$10 μm focal depth. This mechanism eliminates the need for manual focusing and significantly speeds up the image capturing process. Custom software (GenoSensor, Vysis, IL) operating either in the Macintosh or Windows NT environment was used for image acquisition and data analysis.[7]

2.2 Substrate

The background signal from several substrate materials was compared using the imaging system described above. Standard soda lime glass microscope slides were purchased from VWR Scientific (PA) (48312-400). Fused quartz glass microscope slides were obtained from Structure Probe (PA). Black glass slides (Schott MUG-2 and MUG-6) were supplied by Schott Corporation (NY). Chrome coated soda lime glass plates (with high reflectivity) and aluminum coated soda lime glass plates were obtained from Nanofilm (CA), and cut into

the size of a regular microscope slide (75 mm×25 mm). All slides were treated with concentrated H_2SO_4 and then rinsed with H_2O.

2.3 DNA Attachment and DNA Chip Fabrication

The DNA preparation and arraying process is reported elsewhere.[8] In short, lambda DNA was purchased from Gibco BRL (Rockville) and all other DNA targets (BAC, PAC, or P1 clones) were extracted from *E. coli* cells and extensively purified to free them from any materials that could produce autofluorescence. A custom *X, Y, Z* robot system was used to generate DNA microarrays by delivering on the order of 0.3 nL denatured DNA solution per spot. The resulting spots averaged 200 μm in diameter with a center to center distance of 300–500 μm. Arrays were made on 25 mm×17 mm chromium chips, which were inserted into molded plastic carriers to facilitate handling and imaging.

2.4 Probe Labeling and DNA Chip Hybridization

Detailed assay protocols are described elsewhere.[9] In brief, probe DNAs were labeled either by nick translation with SpectrumGreen-dCTP (absorption maximum 494 nm, emission maximum 520 nm) or chemically with SpectrumRed (absorption maximum 574 nm, emission maximum 602 nm, both from Vysis). Hybridizations were performed for 18 h at 37°C in a total volume of 25 μL, containing probe DNA mixtures and hybridization buffer (final conc. 44% formamide, 9% dextran sulfate, 1.8× SSC). After hybridization the microarrays were washed twice with 1× SSC (2 min each at room temperature), followed by three washes in 50% formamide/2× SSC at 40°C (10 min each), three washes in 1× SSC (5 min each at room temperature), and a final wash with 1× SSC (several seconds at room temperature). The arrays were then dried and counterstained. DAPI (absorption maximum 358 nm, emission maximum 461 nm, also from Vysis) was used as a counterstain (1 μM DAPI in mounting media, *p*H 8.0–8.5) to facilitate DNA spot segmentation during imaging analysis.

3 Results and Discussion

3.1 Image Analysis Principles

The validity of quantitative fluorescence detection in DNA chip technology is based on the assumption that the background corrected fluorescent signal, I_i, emitted by a target DNA spot *i*, is proportional to the copy number N_i of its complementary sequences in the probe solution. However, the absolute value obtained for I_i is dependent of a large number of variables, which may vary locally even for spots of equal size and DNA content (i.e., repeat spots of the same target clone). The reason is that the exact conformation, concentration, and number of nucleotides available for hybridization may vary from spot to spot. Other local variations may affect the hybridization efficiency, the efficiency of fluorescence generation, or the efficiency of its capture. Indeed, a significant unevenness in the excitation intensity across the captured image is inherent to the specific design of this imaging system. There are many parameters that affect I_i, but for the purpose of this discussion they can be grouped into four categories, whereby I_i is a function of the target, $f(t)$, the hybridization efficiency, $f(h)$, probe parameters other than concentration, $f(p)$, and instrumentation parameters, $f(d)$, i.e.,

$$I_i = f(t_i) \times f(h_i) \times f(p_i) \times f(d_i) \times N_i = k_i N_i, \qquad (1)$$

where k is a constant, dependent on the target spot on a particular chip, the hybridization assay, and the measurement. Therefore, quantitative image analysis would be extremely difficult if it depended on comparison of absolute intensities between various target spots.

On the other hand, using the dual color assay format first described for comparative genomic hybridization (CGH),[10] these problems can be circumvented by comparing intensity ratios rather than absolute intensities.[10–12] For genomic assays this is achieved by labeling the sample or test DNA with a green fluorophor and a reference DNA (typically normal human DNA) with a red fluorophor. Both DNAs are mixed and hybridized simultaneously to the microarray in the presence of unlabeled Cot-1 DNA (to suppress repeat sequences). The fluorescent intensity of each color is then measured, corrected for local background, and the ratio of I^G (green intensity) over I^R (red intensity) is determined, i.e.,

$$\frac{I_i^G}{I_i^R} = \frac{k_i^G N_i^G}{k_i^R N_i^R}. \qquad (2)$$

Under the assumption that local variations on the chip affect hybridization kinetics and instrument efficiencies at the same rate for the green test DNA and the red reference DNA and that the target DNA does not discriminate between the two probes, then

$$\frac{I_i^G}{I_i^R} = K \frac{N_i^G}{N_i^R}, \qquad (3)$$

where K is approximately a constant independent of the target spot for a particular hybridization and a particular measurement, and the ratio of I^G/I^R is directly proportional to the ratio of test and reference DNA molecules in the hybridization solution. The multicolor imaging system described here makes use of this principle, and we refer to this assay as genosensor based CGH, or gCGH.

3.2 Imaging System Characteristics

A xenon arc lamp was chosen since it provides a white light source with a relatively flat spectrum output in the visible range,[13] allowing the use of many different fluorescent dyes. The output beam, collimated with the parabolic reflector, had a divergence of 3.1° for the center portion collected by the spherical mirror. This permitted the interference excitation filters (acceptance angle 10°) to be placed in the beam without further beam reshaping. The irradiance at the DNA chip surface of filtered excitation light for the blue, green, and red dyes were determined to be 22.3, 27.4, and 33.1 mW/cm^2, respectively, using a broadband power meter (13PEM001/J, Melles Griot, CA).

The use of reflective optics in the excitation optical train simplified the design and eliminated chromatic imperfections that are typically associated with lenses, fiber optics, and other refractive optics. This is important for multicolor detection since an identical illumination profile is required for each

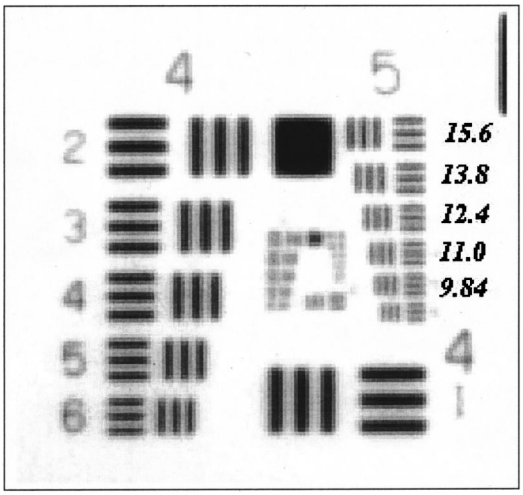

Fig. 2 Image of positive standard USAF 1951 resolution test pattern. Italic numerical values indicate the linewidth for the adjacent elements in group 5.

color. Another advantage for using mirrors is that autofluorescence from the substrate of the optical components is eliminated. The field stop was used to control the illumination field so that only the intended sample area was illuminated, thus reducing the source of background fluorescence and scattering.

The basic design for the collection and imaging optics was adopted from that of Wittrup et al.[6] Two identical camera lenses coupled in a head-to-head configuration provide 1× magnification and a high collection efficiency, which was controlled by opening of the *F* stop of the objective lens (fixed at 2.8 for routine use). The image resolution was tested with a positive standard USAF 1951 resolution test pattern (Edmund Scientific, PA). Lines in group 5 of the image, corresponding to 1–2 CCD pixels, were distinguishable (Figure 2). This indicates that the resolution is limited by pixel size only, and should suffice for DNA spots exceeding a diameter of 50 μm. The effect of chromatic aberration can be minimized by carefully adjusting the focus to maintain the same resolution as that shown in Figure 2 for any fluorescent emission frequency of interest.

Standard gCGH assays typically involve three different fluorochromes. The excitation filters are single-band pass filters matched to the fluorochromes, while a single triple-band pass filter is used in the emission optical train. This filter combination eliminates image shifting due to filter changes. For the green dye, the ratio of signal intensity at 570 nm excitation over signal intensity at 490 nm excitation was found to be 0.0024. For the red dye, the signal intensity ratio at 490 nm excitation over signal intensity at 570 nm excitation was 0.011. The emission of the blue dye at either 490 or 570 nm excitation was negligible. Because of these small ratios no corrections were necessary to account for cross talk between color channels in the data analysis. Since the blue channel is used for segmentation purposes only, the emission of the green and red dyes at 358 nm excitation did not affect the image analysis results.

The beam shape of the collimated output of the lamp is of Gaussian shape[13] and consequently the illumination at the microarray surface is spatially nonuniform. In addition, aberrations of the collection optics also produce a small shading effect in the image. Figure 3 shows the overall shading due to illumination unevenness and imaging aberrations, as measured with a plastic filter (H35136, Edmund Scientific, PA) mounted onto the surface of a chromium coated glass chip. The shading patterns were the same for the green and red channels. A scatter plot (Figure 4) of red intensity versus green intensity for each of the pixels shown in Figure 3 reveals a linear correlation between the intensities for the two colors for all pixels, with a few exceptions most likely due to dust particles or impurities on the plastic filter surface. This fulfills the critical instrument related assumptions underlying Eq. (3).

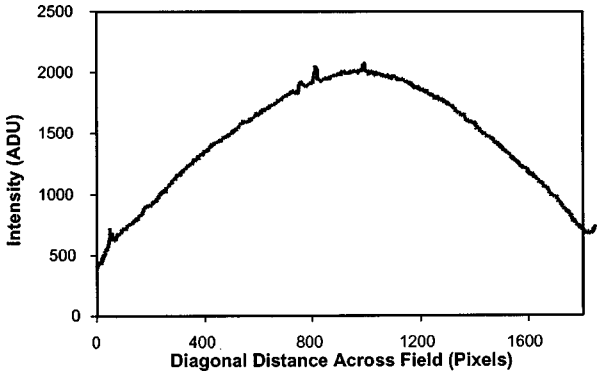

Fig. 3 Overall shading effect due to illumination inhomogeneity and imaging aberrations, showing the pixel intensity along the diagonal line of the image of a plastic filter. The image was acquired with excitation centered at 570 nm.

The detection limit of the imaging system was determined with fluorescent dye (SpectrumRed, Vysis, IL) spots on a thin film (~10 μm) of acrylamide gel. The gel was made on top of the chromium surface of a coated glass chip. Decreasing concentrations of the red fluorescent dye was deposited on the polyacrylamide film. As shown in Figure 5, the detection limit of the system is about 5×10^7 molecules/cm^2 with a signal-to-background ratio of 2. The overall detection efficiency was about 0.01 photoelectrons per molecule per second. With this

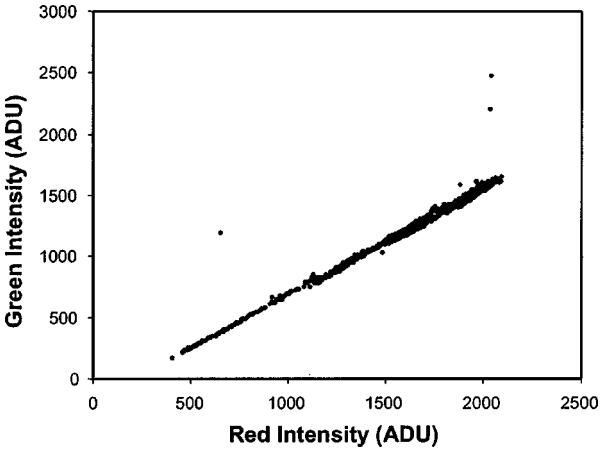

Fig. 4 Correlation plot of pixel intensities for red and green color channels. The data points are the same as in Figure 3.

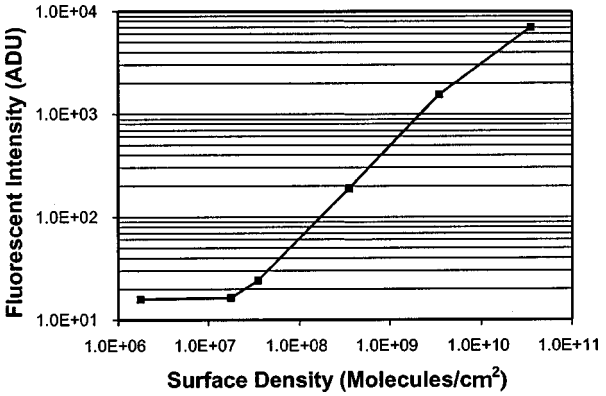

Fig. 5 Plot of measured fluorescence intensity vs surface density of SpectrumRed molecules.

Table 1 Relative background autofluorescence of substrates.

Substrate material	405 nm excitation	490 nm excitation	570 nm excitation
Soda lime	1	1	1
Fused quartz	0.13	0.12	0.15
Schott M-UG-2 black glass	0.11	0.08	0.14
Schott M-UG-6 black glass	0.10	0.08	0.14
Soda lime with Cr coating	0.03	0.02	0.03
Soda lime with Al coating	0.03	0.02	0.03
Silicon wafer	0.08	0.06	0.08

sensitivity and signal-to-background ratio, at low fluorophor surface density, the detection signal-to-noise (S/N) ratio was largely determined by CCD readout noise. For a spot with diameter of 100 μm and surface density of 3 molecule/μm^2, the detection S/N ratio with 10 s integration was about 14.5, nearly five times the S/N ratio of a confocal DNA array scanning system.[14] The image acquisition time for a typical hybridized genomic DNA array was 20 s for the two analytical color channels and 5 s for the counterstain channel.

3.3 Substrate Surface

DNA chip based assays require the detection of weak fluorescence signals. Most fluorescence assays are carried out on soda lime or borosilicate glasses (standard microscope slides), containing impurities that can produce fluorescent emissions (autofluorescence) throughout the visible region.[6] With the imaging system described above the autofluorescence of a typical soda lime glass microscope slide was determined to be equivalent to the signal from a layer of red dye with a surface density about 1×10^9 fluorophors/cm^2 (data not shown). This amount of background signal together with noise from other sources, such as stray light and Rayleigh and Raman scattering, can obscure the detection of weak signals. An effective methods to reduce substrate autofluorescence is the use of confocal optics and scanning to read the arrays, which relies on the high *z*-axis resolution to reject out of focal plane emissions. For the system design described herein, the same method cannot be implemented to limit fluorescence emitting from the substrate. We have therefore investigated different substrate materials as an alternative approach to reduce substrate autofluorescence. The values listed in Table 1 are calculated with dark frame corrected images and are scaled to the autofluorescence of a soda lime glass microscope slide. Any potential contributions from Rayleigh scattering, Raman scattering, filter imperfections, stray light, or other sources of background fluorescence were included.

Although quartz offers a nearly 10-fold improvement in autofluorescence reduction over soda lime glass the cost is prohibitive. A similar effect could be achieved by reducing the thickness of the soda lime substrate 10-fold, however, the demands on durability during manufacturing, shipping, and handling in the hybridization, etc., makes this approach impractical. A significant problem for any transparent material is dust particles or other contaminants on the underside of the slide, which may produce very intense fluorescent spots. Since they are out of focus, they may appear in the image of equal size as DNA spots, complicating the segmentation and image analysis process. Nontransparent materials offer an attractive solution since they adsorb a large fraction of the excitation light, as well as most of the light emitted from the substrate or from below. The best solution seems to be offered by slides coated with a metallic surface, which completely eliminate the penetration of excitation light into the substrate, or the passage of any light from below the substrate surface.

Background suppression is only one of the criteria for choosing a substrate material. The substrate must also be chemically and mechanically compatible with the intended use, and easily available at low cost. For these reasons we have chosen chromium coated glass for our microarrays. While the chromium coating is inferior in reflectance (50%) to some other metals (Al and Ag, for example, exceed 90%), it is superior in other important characteristics. Like aluminum, chromium oxidizes when exposed to air, and the resulting oxide layer is extremely resistant to oxidizing agents, such as sulfuric acid. Its hardness provides scratch resistance, and with appropriate treatment the surface can be made highly hydrophobic.[15,16] A hydrophobic surface is advantageous for forming small target spots when depositing the DNA, and results in significantly less background due to nonspecific binding of probe molecules during the hybridization. Because it is widely used in photolithography, chrome coated glass is easily available and relatively inexpensive. A direct comparison of chromium coated slides to conventional microscope slides was performed with fluorescein conjugated beads (Flow Cytometry Standards Corp.). These beads were suspended in a commonly used antifade medium and sandwiched between the substrate and a glass coverslip. The prepared slides were then imaged with the imaging system. Quantitative analysis of the images revealed that the overall background for the chromium coated slide is only 21% that for regular glass, while the net signal intensity is about double (data not shown). Note that in this analysis the overall background included scattering, stray light, filter imperfection, mounting medium, and coverslip contributions.

3.4 DNA Attachment on a Chromium Surface

Several attachment methods have been developed for immobilizing target DNAs. Most involve chemically functionalizing the support surface to provide an active group, such as an amino group, to form ionic or covalent bonds between the surface and the DNA, either directly[11,17,18] or after introducing a reactive group into the DNA.[19] In an effort to develop methodologies for attaching DNA to a chromium surface we tested the surface activation of chromium with glycidoxipropyltrimethoxysilane (GPTS), as described for glass supports.[20] In order to insure efficient silanization, the chromium surface was first treated with a 2% water-based solution of silesquioxane oligomers (Gelest, Inc., Tullytown, PA) for 10 min at room temperature, followed by a water wash. DAPI staining was used as a measure to test attachment of target DNA. The ability of attached DNA to participate in the hybridization reaction was tested with a fluorescently labeled probe. With regard to either of these parameters no difference was observed between chromium coated glass before and after treatment with GPTS, using either aminated or unmodified target DNA. However, unmodified DNA had to be larger than 300 nucleotides in length and denatured before attachment was sufficiently strong to withstand the hybridization and washing conditions. Typical attachment conditions involve deposition of about 300 pL DNA (1 μg/μL) in NaOH (100 mM).

3.5 Dynamic Range of the Assay System

The two-color comparative hybridization system described above for the gCGH assay is also used for the measurement of expression profiles,[12] whereby mRNA from one tissue (e.g., cells before drug treatment) is compared to the mRNA from a second tissue (e.g., the same cells after treatment). For this the mRNAs are converted to cDNAs by reverse transcription and simultaneously labeled with different fluorochromes. Since the expression of genes can vary from 1 copy per cell to thousands of copies per cell, a dynamic range of the assay system of at least four logs is desirable. Figure 6 shows a simulation of such a situation using green and red labeled lambda DNA. Between 25 ng and 2.5 pg of green DNA was mixed with 250 pg red DNA and hybridized to a microarray containing lambda DNA targets on the perimeter of the array area, where excitation intensity is at a minimum. A linear dose response in the green/red ratio was obtained over the whole concentration range, indicating that our imaging system combined with the chromium substrate should be suitable for expression analysis. In genomic applications, where amplifications of sequences from the normal copy number of 2 to more than a thousand are quite rare, a 12 bit camera with a pixel depth of only three logs is sufficient, which is the current system configuration. Thus, two separate images with different exposure times had to be taken to test the total dynamic range of this system. It is noteworthy that the standard deviation of the green/red ratios of all 80 lambda spots is less than 5%, further indicating that shading and optical imperfections are negligible for the purpose intended.

3.6 Detection of Oncogene Amplification by gCGH

Figure 7 shows a typical application of the gCGH technology to the genomic analysis of tumor cells. Colo320, a cytogenetically reasonably well characterized tumor cell line (ATCC

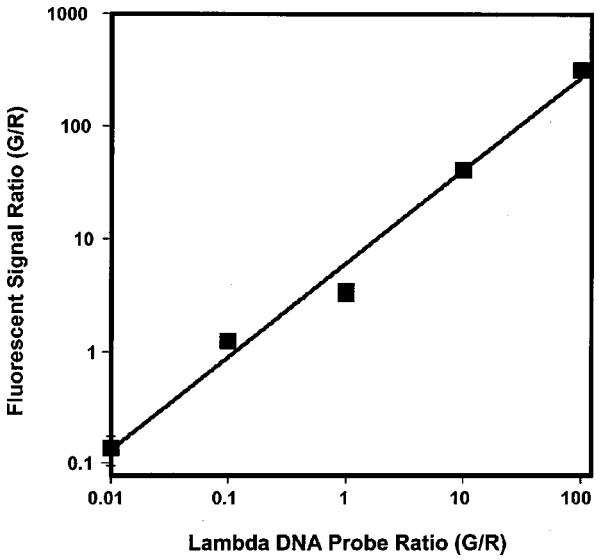

Fig. 6 Dynamic range of the assay system. Denatured double-stranded lambda target DNA was deposited on chromium chips (80 spots per array). Lambda probe DNA was labeled by nick translation. Each hybridization reaction contained a mixture of 250 pg of SpectrumRed lambda DNA and either 2.5 pg, 25 pg, 250 pg, 2.5 ng, or 25 ng of SpectrumGreen lambda DNA. The average green/red intensity ratios are plotted vs the concentration ratio of green/red probe DNA.

No. CCL-220.1) was chosen as source for the test DNA, since it has an approximately 29-fold amplification of the chromosomal region harboring the cMyc gene. Colo320 DNA (0.4 μg, labeled green) and 0.25 μg of chemically labeled (red) normal human DNA were mixed with 100 μg of Cot-1 DNA. After hybridization (18 h, 37 °C) to a microarray containing 52 different chromosomal targets (each with 3 repeat spots) the chips were washed and imaged. The background corrected intensity ratios for the test/reference color plane were obtained from the Genosensor Image analysis software.[7] Assuming that most target loci are present in the normal copy num-

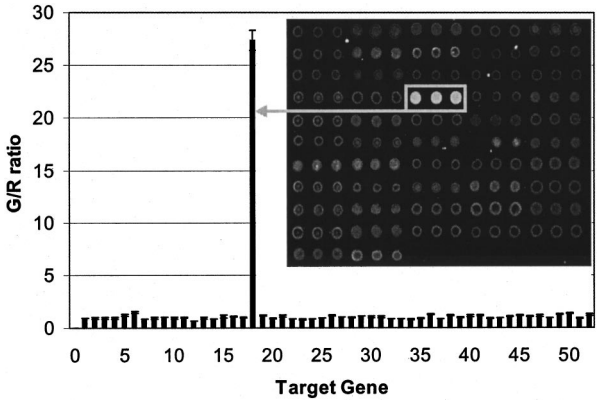

Fig. 7 Detection and quantitation of cMyc amplification in the tumor cell line Colo320. A microarray containing 52 genetic loci was cohybridized with 400 ng of green Colo320 DNA and 250 ng of red human reference DNA. The composite image is shown in the inset. The box indicates the 3 cMyc target spots. Normalized test/reference intensity ratios (yellow) with standard deviations between repeat spots are plotted for each target sequence.

ber of 2 in the Colo320 DNA (not quite correct for this cell line) then the median test/reference ratio of all spots can be used to normalize all ratios. In the experiment shown in Fig. 7, the normalized test/reference ratios for all targets except cMYC was on the order of 1.0 with a standard deviation of 0.16. Important is the fact that the standard deviation and the coefficient of variation for repeat spots were less than 10% for most targets. The test/reference ratio for cMYC was found to be 27, consistent with the expected amplification level for this gene. For unknown samples the normalization process is of course very critical and this is described elsewhere.[21,22]

4 Conclusion

The combination of the chromium surface with the CCD-based imaging system described herein provides a highly sensitive and quantitative assay system for DNA microarrays. Assay sensitivity is achieved through reduction in autofluorescence and nonspecific probe binding, i.e., by reducing the background rather than the increasing specific signal intensity. The linear dose response over four orders of magnitude of the assay system should suffice for both genomic and expression studies, but should also provide the basis for other fluorescence based analytical applications, such as protein arrays and immunoassays. The use of chromium coated glass as a solid support for DNA chips is only one embodiment of the method for background reduction in fluorescence based assays. Other coating and substrate materials can be used as alternatives provided that they are mechanically, physically, and chemically compatible with the particular assay.

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