*Anal. Chem.* **2010,** *82,* 9901–9908

# Fluorescently Imaged Particle Counting Immunoassay for Sensitive Detection of DNA Modifications

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**Modifications of genomic DNA may change gene expression and cause adverse health effects. Here we for the first time demonstrate a particle counting immunoassay for rapid and sensitive detection of DNA modifications using benzo[*a*]pyrenediol epoxide (BPDE)−DNA adducts as an example. The BPDE-adducted DNA is specifically captured by immunomagnetic particles and then isolated from unmodified DNA by applying an external magnetic field. By taking advantage of the fluorescence signal amplification through multiple labeling of captured DNA by OliGreen dye, the captured BPDE−DNA adducts can be quantified by particle counting from fluorescence imaging. This clearly demonstrates that the number of fluorescently countable particles is proportional to the modification content in genomic DNA. It is interesting to note that the background fluorescence signal caused by nonspecific adsorption of OliGreen dye can be more effectively quenched than that induced by the binding of OliGreen dye to ssDNA, allowing for significant reduction in the background fluorescence and further enhancing the detection sensitivity. The developed method can detect trace BPDE−DNA adducts as low as 180 fM in the presence of 1 billion times more normal nucleotides in genomic DNA and has a dynamic range over 4 orders of magnitude. By using anti-5-methylcytosine antibody, the method is extended to the detection of global DNA methylation. With high sensitivity and specificity, this rapid and easy-to-perform analytical method for DNA modifications shows a broad spectrum of potential applications in genotoxical and epigenetic analysis.**

Magnetic micro- and nanoparticles have wide applications in bioanalysis, including aptamer selection, proteomics research, and clinical diagnostics, due to their unique separation power endowed by their paramagnetic property.[1,2] When functionalized with specific recognition elements (e.g., affinity substrates, aptamers, DNA, and antibodies), magnetic particles can be used for specific capture of desired analytes from a complex biological matrix,[3,4] including environmental pollutants,[5] various nucleic acids,[6,7] aptamers,[8,9] phosphopeptides,[10−12] cancer marker proteins,[13−15] and pathogenic bacteria.[16−18] The captured targets can be detected using appropriately designed signal probes, such as quantum dots,[19] carbon nanotubes,[20] Au nanoparticles,[21] and liposomes encapsulated with fluorescent dye.[22] Although some of the designed signal probes may provide an amplified signal, it is still a challenge to quantitate targets simply by particle counting. Despite the complex design and versatile functions, routine flow cytometry based particle counting analysis cannot distinguish specific binding from nonspecific adsorption and is only suitable for particles with a size over micrometers.

Here we for the first time demonstrate a particle counting immunoassay for rapid and sensitive detection of DNA modifica-

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Published on Web 11/09/2010

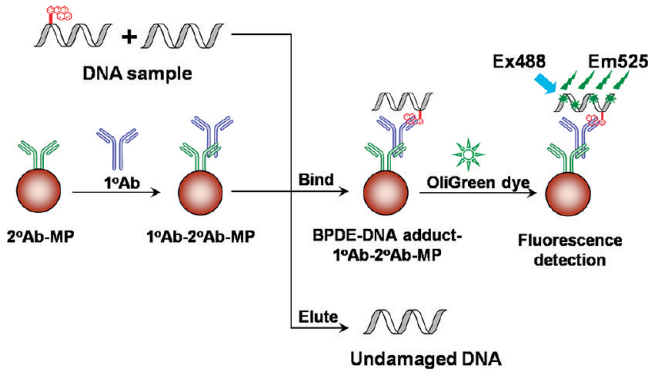


**Figure 1.** Schematic illustration of the immunodetection of DNA modification by magnetic separation combined with particle counting. The MPs (magnetic particles) with captured ssDNA that is multiply stained by OliGreen dye can be counted under fluorescence microscopy, but not the MPs without captured ssDNA.

tions. The approach is illustrated in Figure 1. In cells, genomic DNA can be modified by oxidative stresses and various carcinogens, leading to a permanent change in DNA if the lesions are incorrectly bypassed by biological repair systems. These modifications are biomarkers of the exposure to carcinogens and useful for human biomonitoring.[23,24] On the other hand, genomic DNA can also be normally modified under physiological conditions as the methylation of position 5 of cytosine (DNA methylation), which has been exploited by eukaryotic organisms as a primary epigenetic mechanism for regulation of gene expression, embryogenesis, and reproductive development.[25,26] To specifically capture the modified DNA, magnetic particles were functionalized with a secondary antibody which can bind to the primary antibody with high affinity. The noncovalently immobilized primary antibody can specifically recognize the modification in genomic DNA, allowing efficient and specific capture of the modified DNA. Although trapped on magnetic particles, the modified DNA cannot directly provide a signal for detection. By staining with an ssDNA dye, the modified DNA molecules can then be sensitively detected under fluorescence imaging. In this approach design, the captured targets can be transduced into an amplified fluorescent signal by multiple staining of the switch fluorescent dye upon binding to DNA, providing the possibility of particle counting for quantification of DNA modification.

## EXPERIMENTAL SECTION

*Caution: anti-BPDE is carcinogenic and should be handled with extreme caution!*

**Chemicals and Reagents.** *anti*-Benzo[*a*]pyrene-7,8-dihydrodiol 9,10-epoxide (*anti*-BPDE) was supplied by the National Cancer Institute Chemical Carcinogen Reference Standard Repository (Kansas, MO). Mouse monoclonal anti-BPDE−dG antibody was purchased from Trevigen (Gaithersburg, MD), and mouse monoclonal anti-5-methylcytosine antibody was from Calbiochem (La Jolla, CA). BioMag goat anti-mouse IgG magnetic particles were from Polysciences (Warrington, PA). OliGreen ssDNA reagent was from Invitrogen (Carlsbad, CA). λDNA was from Sigma (St. Louis, MO). The oligonucleotide 6-carboxylfluorescein (FAM) labeled 16-mer with a sequence of 5′-TCCATTATGCATAACC was purchased from Shanghai Sangon Biological Engineering Technology & Services Co., Ltd. (Shanghai, China), and the ninth base guanine (counted from the 5′ end) was further modified with *anti*-BPDE following the protocol described previously.[27] *Escherichia coli* CpG methyltransferase M. *Sss* I was from New England BioLabs (Ipswich, MA). Fetal bovine serum was supplied from Gibco BRL (Gaithersburg, MD), and other reagents used for cell culturing were from Hyclone (South Logan, Australia). Deionized water (18.2 MΩ·cm) was obtained from a Purelab Ultra Bioscience water purification system (ELGA, U.K.). All other reagents and solvents were of analytical or HPLC grade.

**Preparation and Quantification of BPDE−DNA Adduct Standard.** BPDE−DNA adduct standard was synthesized by the reaction of *anti*-BPDE with human genomic DNA, which was isolated from cultured A549 cells.[28] To calibrate the content of BPDE−DNA adducts in treated genomic DNA, one aliquot of genomic DNA was enzymatically digested into individual deoxynucleotides and *anti*-BPDE−$N^2$-dG was quantified by using stable isotope dilution ultraperformance liquid chromatography−tandem mass spectrometry (UPLC−MS/MS) as described previously.[28] The estimated total frequency of BPDE−DNA adducts in genomic DNA standard was 6.0 adducts/$10^5$ nucleotides. This BPDE−DNA adduct standard was used for further magnetic particle based particle counting immunoassay.

**Cell Treatment by *anti*-BPDE.** Human lung carcinoma A549 cells were grown in 4.0 mL/plate RPMI 1640 medium supplemented with 10% fetal bovine serum, 100 U/mL penicillin, and 100 U/mL streptomycin at 95% humidity and 5% $CO_2$ for 2−3 days prior to use. The cultured A549 cells (about $5 \times 10^6$ cells/plate) were treated for 2 h by *anti*-BPDE of different doses (0, 1, 10, and 100 nM). *anti*-BPDE was freshly prepared in tetrahydrofuran/triethylamine solution (THF/TEA; 19:1, v/v) and immediately used for cellular treatment. After being washed by phosphate-buffered saline (PBS) twice, genomic DNA was extracted from treated A549 cells using Wizard genomic DNA purification kits (Promega, Madison, WI), following the protocol of the manufacturer. The DNA concentration was estimated by measuring the UV absorbance at 260 nm. One aliquot of genomic DNA (3 μg) was used for detection of BPDE−DNA adducts using magnetic particle based particle counting immunoassay.

**Detection of BPDE−DNA Adducts.** BioMag goat anti-mouse IgG magnetic particles (5 μg, $5 \times 10^6$ particles) were first washed with 10 mM phosphate buffer (PB; pH 7.4) three times and then incubated with 2 μg of anti-BPDE−dG antibody at 4 °C for 30 min. The optimization of the incubation time is described in the Supporting Information (Figure S1). Magnetic particles were collected and isolated from unbound primary antibody by an external permanent magnet and resuspended in 20 μL of PB. The DNA samples (20 μL) were denatured by heating at 95 °C for 10 min and then chilled on ice. The magnetic particles with immobilized anti-BPDE−dG antibody (2 μL, $5 \times 10^5$ particles) were added to each denatured DNA sample. The reactions were carried out for 30 min at 4 °C with gentle swirling of the

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reaction vials at 10 min intervals to keep the particles in suspension. The magnetic particles with bound BPDE−DNA adducts were separated by the external magnet. The magnet was placed near one side of the sample tube; then the supernatant was carefully discarded, and the magnetic particles were left in the tube. The left magnetic particles were then washed with PBS three times to remove unbound DNA. Finally, BPDE−DNA adducts captured on magnetic particles were stained for 5 min with fluorescent dye OliGreen (diluted as 1/800 in 10 mM Tris−HCl, 1 mM EDTA, pH 7.5). The optimization of the staining concentration of OliGreen is described in the Supporting Information (Figure S2). The collected magnetic particles were transferred into wells of 6 mm × 6 mm set by adhesive papers on cover glasses. Then the stained magnetic particles were irradiated by blue light (450−490 nm) at a relative power of 10% from a short arc lamp (100 W, Osram, Germany) equipped in a Leica TCS SP5 laser scanning confocal microscope (Germany). This prequenching step can effectively reduce the fluorescence interference from adsorbed OliGreen dye. After 30 s of prequenching, the magnetic particles in the wells on the cover glasses were fluorescently imaged using the laser scanning confocal microscope.

**Detection of Global DNA Methylation.** To carry out in vitro methylation of cytosine in the CpG dinucleotides, λDNA (5 μg) was treated by CpG methyltransferase M. *Sss* I (20 U) for 4 h at 37 °C according to our recent work.[29] The content of methylcytosine in methylated λDNA was about 1 in 20 nucleotides. The global DNA methylation level in methylated λDNA and genomic DNA was analyzed by magnetic particle based particle counting immunoassay similar as that for the detection of BPDE−DNA adducts. The unmethylated λDNA (50 μg/mL) was used as a negative control. DNA samples were denatured by heating at 95 °C for 10 min, followed by chilling on ice for 10 min, and then mixed with anti-5-methylcytosine antibody conjugated magnetic particles (2 μL, $5 \times 10^5$ particles). After 30 min of incubation, the magnetic particles were washed three times with PBS and then stained with OliGreen for fluorescent imaging.

**Image Acquisition and Particle Counting Analysis.** Fluorescence images were taken by using a laser scanning confocal microscope setup consisting of a Leica DMI 6000B inverted microscope (63× objective, 1.40 NA) and a Leica TCS SP5 confocal scanning system. The fluorescence of ssDNA−OliGreen complexes was excited by the 488 nm line of an argon ion laser (4.5 mW), and the emission was detected by a photomultiplier tube (PMT) at 500−550 nm. The PMT voltage was set at 800 V. The bright-field images were acquired at the same time with a PMT gain of 280 V. Each image was taken from a physical dimension of 246 μm × 246 μm. The analysis of the fluorescent dots was carried out by using a free image processing program, ImageJ, developed by the National Institutes of Health.[30] The fluorescent dots in the images were counted by setting a threshold at a pixel value of 50 (8 bit image) to exclude the particles with a fluorescence signal comparable to the background fluorescence. The fluorescent intensity of each dot was measured by using the gray value in the range of 50−255. The brighter dots with gray values above 255 were also counted as having a gray value of 255.

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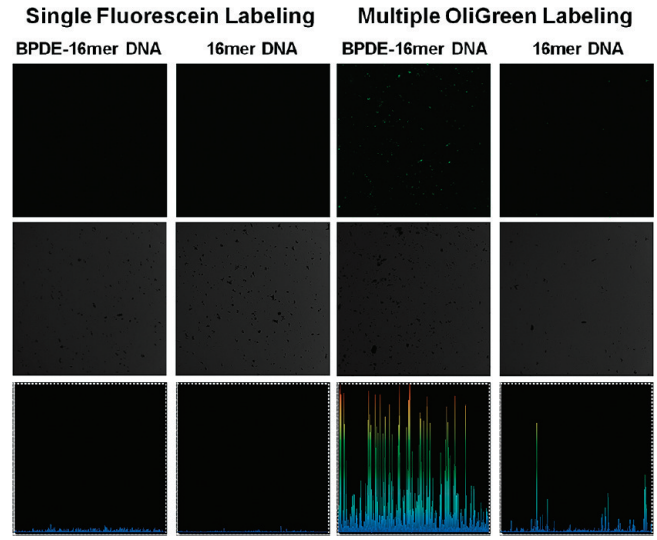


**Figure 2.** Comparison of multiple labeling with single labeling for particle counting analysis of short 16-mer oligonucleotide adducts. The fluorescent images, bright-field images, and side views are shown from top to bottom.

Then the "Analyze Particles" function of ImageJ was used to count the number of fluorescent dots in each image. The average number and standard deviation were obtained by counting randomly at least 10 images for each sample.

## RESULTS AND DISCUSSION

**Particle Counting by Multiple Labeling of Short Oligonucleotide Adducts.** To illustrate particle counting for the immunodetection of DNA modifications, a short oligodeoxynucleotide (oligo), a 6-carboxylfluorescein (FAM)−BPDE 16-mer, was first used as an example. This short oligo was modified by a single BPDE−dG adduct in the middle of the chain and fluorescently labeled at the 5′ end by a single tag of FAM. Magnetic particles conjugated with goat anti-mouse IgG were used to immobilize mouse monoclonal anti-BPDE−dG antibody (mAb 8E11), which can specifically recognize single *anti*-BPDE−$N^2$-dG adducts in oligonucleotides and genomic DNA with high affinity and negligible cross-reaction.[31] Due to high-affinity recognition against BPDE−dG adducts, the immobilized mAb 8E11 on magnetic particles can specifically capture the FAM−BPDE 16-mer from the aqueous solution. Since the FAM labeled on the 16-mer has a high quantum yield (∼0.93) and a high extinction coefficient (>75000 $M^{-1}$ $cm^{-1}$),[32] it is reasonable to believe that the captured BPDE 16-mer can generate a strong fluorescence signal. However, even with incubation with excess FAM−BPDE 16-mer, no particle can be observed and clearly imaged using confocal fluorescence microscopy (Figure 2), similar to that incubated with excess control FAM 16-mer without BPDE−DNA adducts. By mapping the fluorescent intensity and position of every dot in the imaged field using Leica LAS AF software, the fluorescent signal of the particles should be more clearly displayed as a side view image. Even with such mathematic treatment, there is no significant difference in the fluorescence

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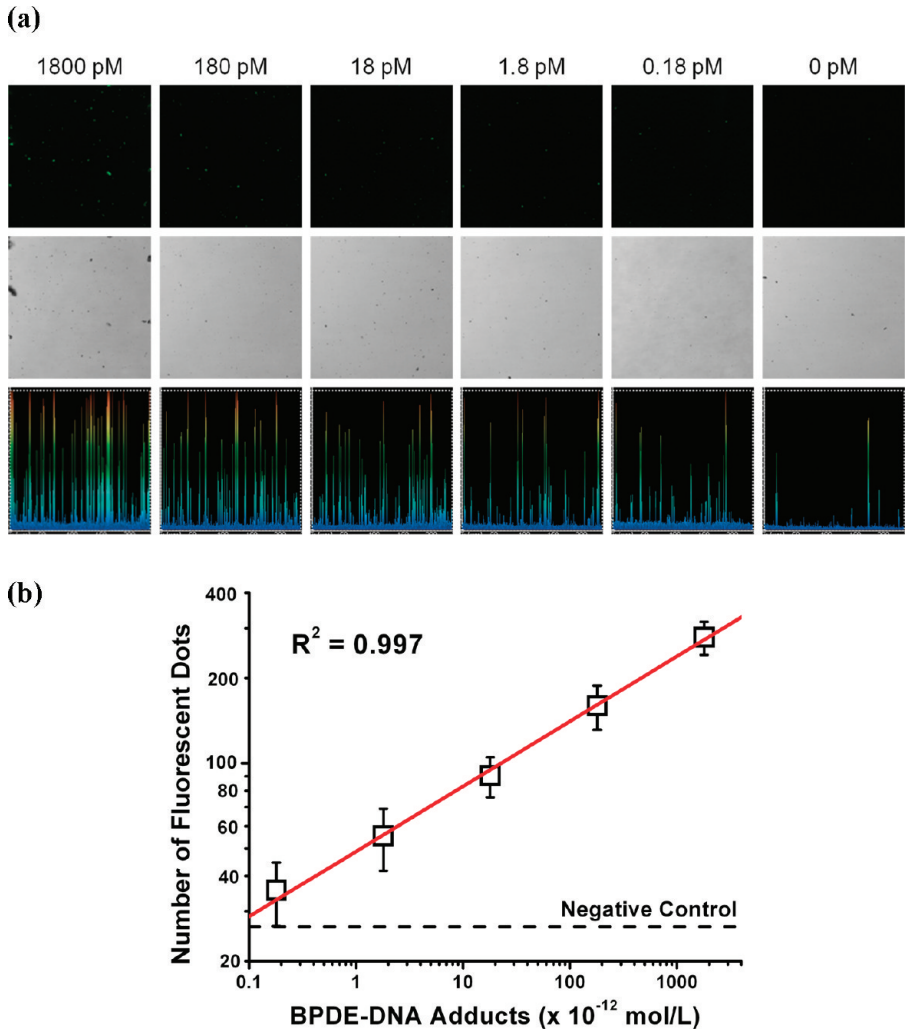


**Figure 3.** (a) Representative fluorescent images (top), bright-field images (middle), and side views (bottom) for detection of BPDE−DNA adducts by particle counting immunoassay based on magnetic separation. (b) Linear relationship between the number of fluorescent dots and the concentration of BPDE−DNA adduct standard. Various concentrations of BPDE−DNA adduct standard (0.18−1800 pM) were mixed with untreated genomic DNA (100 $\mu$g/mL) as DNA samples. The total sample volume was 20 $\mu$L.

signal between the two 16-mers. It is evident that the single labeling with a bright fluorescent dye fails to provide a strong enough signal for possible particle counting.

To amplify the fluorescence signal for possible particle counting, OliGreen dye, an unsymmetrical cyanine compound with a negligible intrinsic fluorescence but 1000-fold fluorescence enhancement and a high quantum yield (~0.95) upon binding to ssDNA,[33] was used for multiple labeling of captured ssDNA molecules. Compared with single labeling, the fluorescent intensity of particles captured with FAM−BPDE 16-mer was greatly enhanced after staining with OliGreen, and the particles become visible under confocal fluorescence microscopy. A number of particles can be clearly imaged as shown in Figure 2 (bottom), demonstrating the feasibility of particle counting. In contrast, much less particles can be observed under the same imaging system when the antibody-functionized magnetic particles of the same amount are incubated with excess control oligo, the FAM 16-mer. The observation of fluorescently imaged particles for the control oligo is probably attributed to the nonspecific absorption of OliGreen and/or OliGreen-stained ssDNA on the surface of the magnetic particles. Nonetheless, it is clear that the presented particle counting immunoassay can differentiate BPDE−oligo adduct from the control oligo through multiple labeling of captured oligo by OliGreen.

**Particle Counting Immunoassay for Detection of BPDE Adducts in Genomic DNA.** The isolated genomic DNA from A549 cells was estimated by 0.8% agarose gel electrophoresis and is about 48 kb. Therefore, the genomic DNA molecules are about 3000 times longer than the short 16-mer. It is expected that the multiple staining of one genomic DNA molecule by OliGreen should be 3000 times brighter than that of one short 16-mer molecule, leading to much higher sensitivity. The hypothesis was first tested using a BPDE−DNA adduct standard. This adduct standard was prepared and characterized according to our previous work[28] and has the same size as that of genomic DNA and a total adduct frequency of 6.0 BPDE−dG adducts/$10^5$ nucleotides. By mixing the adduct standard (0−1.0 $\mu$g/mL) with constant untreated genomic DNA (100 $\mu$g/mL), a series of DNA samples containing 180 fM−1.8 nM BPDE−dG adducts were prepared. Untreated genomic DNA of the same concentration was used as a negative control. Compared with the

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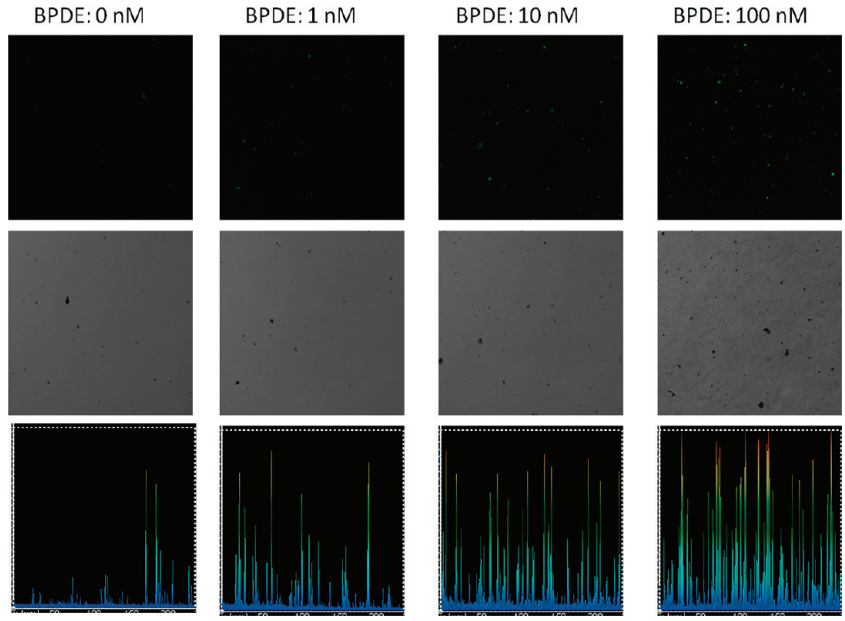


**Figure 4.** Representative fluorescent images (top), bright-field images (middle), and side views (bottom) for detection of BPDE−DNA adducts in A549 cells dosed with 0, 1, 10, and 100 nM *anti*-BPDE by particle counting immunoassay. The final concentration of genomic DNA isolated from A549 was 150 $\mu$g/mL, and the total sample volume was 20 $\mu$L.

negative control, a large number of fluorescent dots can be observed for the magnetic particles incubated with the mixed genomic DNA samples in a concentration of BPDE adduct dependent manner (Figure 3a, top row). As clearly shown in side view images (Figure 3a, bottom row), the number of observed fluorescent dots also increases with increasing concentration of BPDE−DNA adducts. Each fluorescent dot may represent one magnetic particle bound with stained DNA (not limited to one DNA molecule as described in the discussion of the binding stoichiometry). However, the number of observed magnetic particles in the fluorescence-independent bright-field image is the same (about 350 particles per image) for all DNA samples (Figure 3a, middle row). These results confirm that the observed difference in the number of fluorescent dots is related to the presence of BPDE−dG adducts in genomic DNA rather than the random loss of magnetic particles during the immunoreaction, washing, and staining steps.

On the basis of particle counting (performed using ImageJ software), it was found that the number of fluorescent dots increases proportionally to the concentration of BPDE−DNA adduct standard (from 0.18 to 1800 pmol/L) on a logarithmic scale (Figure 3b). The dynamic range is 4 orders of magnitude with a good correlation coefficient of $R^2 = 0.997$ ($n = 5$). The number of fluorescent dots for 180 fM adducts (35.6 ± 9.0, $n = 10$) is statistically different from that of the negative control (28.8 ± 5.3, $n = 10$), judged by the $t$ test ($P < 0.05$). Therefore, the estimated low limit of detection for BPDE−DNA adducts is about 180 fM in concentration and is about 6.0 adducts/$10^{10}$ nucleotides in frequency by accounting for the total DNA concentration of 100 $\mu$g/mL (about 0.3 mM nucleotides). The achieved frequency sensitivity is comparable with that of widely applied and sensitive $^{32}$P-postlabeling assays.[34] Instead of measuring the total fluorescence as in solid-phase immunoassays, we counted the number of fluorescent dots in the images, and the estimated number was used for quantification of BPDE−DNA adducts in genomic DNA. Therefore, we named the developed method "particle counting immunoassay".

**Table 1. Number of Fluorescent Dots and the Frequency of BPDE−DNA Adducts for A549 Cells Treated with *anti*-BPDE (0−100 nM) ($n = 10$)**

| *anti*-BPDE concn (nM) | number of fluorescence dots | number of BPDE−DNA adducts/$10^9$ nucleotides |
|---|---|---|
| 0 | 22.5 ± 2.7 | 0.1 ± 0.0 |
| 1 | 40.9 ± 12.3 | 3.1 ± 0.9 |
| 10 | 76.7 ± 14.0 | 24.3 ± 8.0 |
| 100 | 128.8 ± 26.3 | 311.2 ± 38.9 |

Benefitting from the high sensitivity of the method, it is feasible to detect the trace BPDE−DNA adducts in cultured human lung carcinoma A549 cells exposed to *anti*-BPDE at low doses. The extracted genomic DNA (150 $\mu$g/mL, 20 $\mu$L) is directly subjected to particle counting immunoassay without enzymatic digestion. The estimated number of fluorescent dots (a reflection of the content of BPDE−DNA adducts in genomic DNA) increases with increasing exposure dose of *anti*-BPDE (Figure 4 and Table 1), exhibiting an excellent linear dose−response with a correlation coefficient of $R^2 = 0.999$ ($n = 4$). By using the developed method, we are able to detect the trace BPDE−dG adducts (3.1 ± 0.9 adducts/$10^9$ nucleotides) in A549 cells treated by 1 nM anti-BPDE (Table 1), which is relevant to environmental exposure levels.

In general, the frequency of BPDE−DNA adducts in genomic DNA is very low, and the average number of BPDE adducts in one single-stranded DNA molecule (48 kb) is often less than 1. Therefore, in most cases, one DNA strand cannot bind two or more magnetic particles. Since one magnetic particle may have several conjugated antibody molecules to bind to BPDE−DNA adducts, it is possible for one magnetic particle to bind several DNA strands. The significant variance in fluorescence intensity

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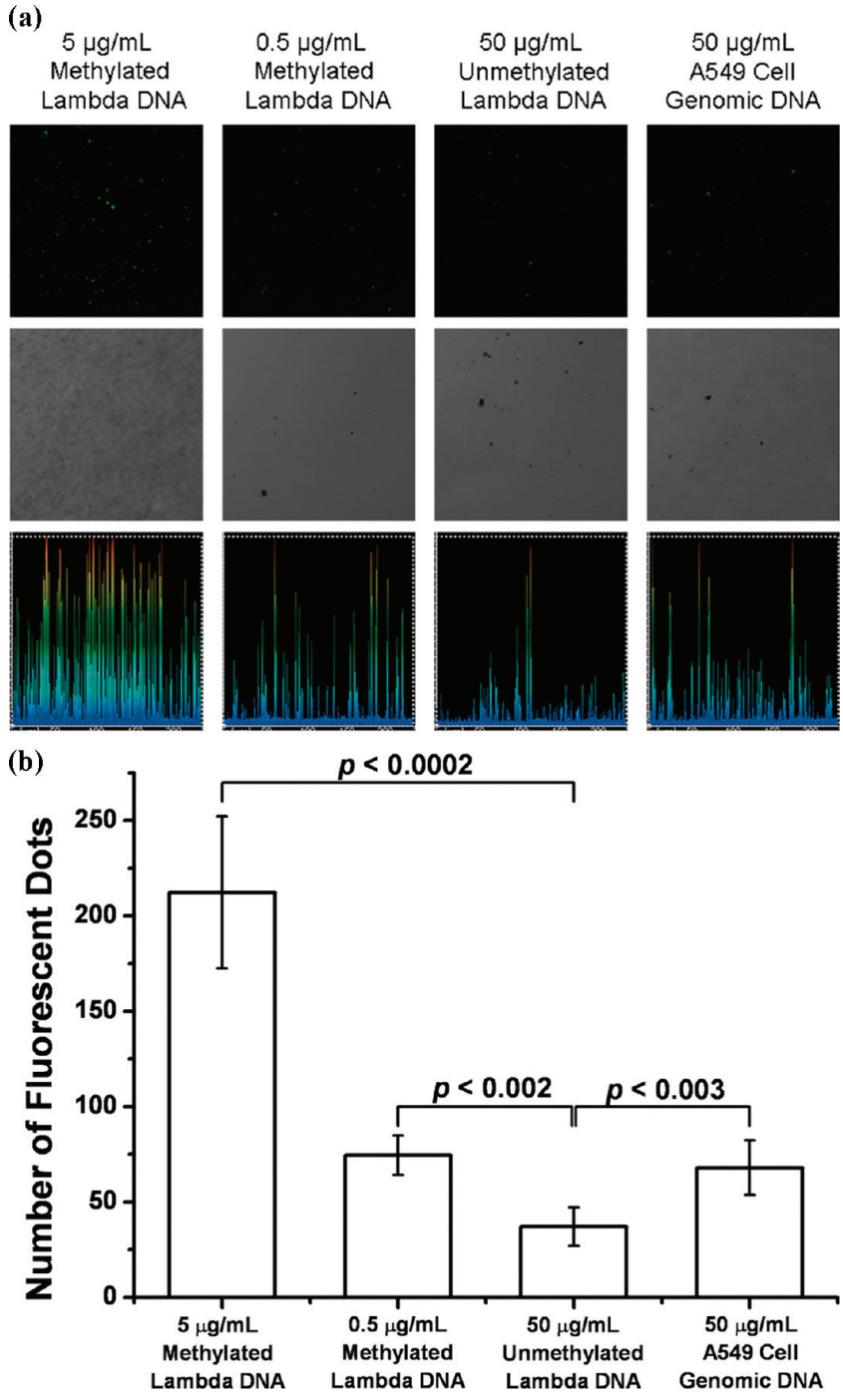


**Figure 5.** (a) Representative fluorescent images (top), bright-field images (middle), and side views (bottom) for detection of global DNA methylation by particle counting immunoassay. (b) Particle counting of the number of fluorescent dots for the detection of global DNA methylation. The total sample volume was 20 $\mu$L.

of the fluorescent dots (side views in Figure 3) may reflect the difference in the binding stoichiometry or length of captured genomic DNA. However, the exact binding stoichiometry is not clear yet.

**Particle Counting Immunoassay for Detection of Global DNA Methylation.** The developed particle counting immunoassay can be extended to the detection of other DNA modifications if the modification-specific antibodies are available. Here we demonstrate the applicability of the method to the analysis of global DNA methylation using anti-5-methylcytosine antibody as a specific antibody. DNA methylation, postreplication modification on the 5 position of cytosine in the context of CpG dinucleotides, can regulate gene expression patterns and ultimately influence the fate of the cell.[25,26] Therefore, DNA methylation plays a crucial role in development of many types of cancers and can be used as a biomarker in cancer diagnosis and therapy.[35−37] Using a method similar to that described above, DNA methylation can be detected by particle counting immunoassay. The number of fluorescent dots for methylated $\lambda$DNA (5 and 0.5 $\mu$g/mL) is much higher than

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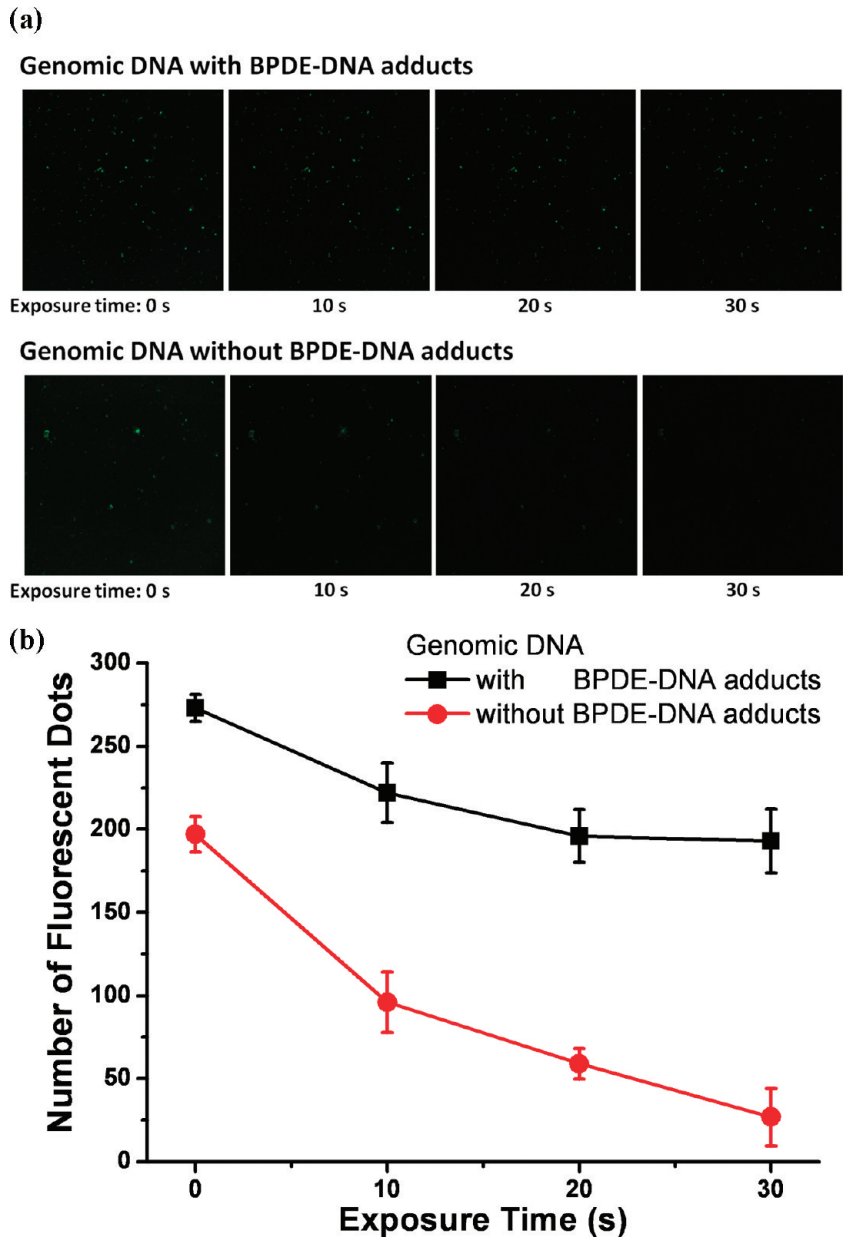


**Figure 6.** (a) Fluorescent images of genomic DNA with or without BPDE−DNA adducts after different exposure times for prequenching. The genomic DNA was captured by antibody-immobilized magnetic particles and stained with OliGreen. (b) Effect of the prequenching protocol for particle counting immunoassay in genomic DNA samples with or without BPDE−DNA adducts. Blue light (450−490 nm) at a relative power of 10% from mercury light (100 W) was used for prequenching. $n = 5$.

that of unmethylated λDNA, even with the latter presented at a higher concentration of 50 μg/mL (Figure 5). Moreover, the number of fluorescent dots associated with DNA methylation increases with increasing concentration of methylated λDNA, showing a clear dose−response relationship (Figure 5b). The genomic DNA extracted from cultured A549 cells shows a significantly higher methylation level than that of the unmethylated λDNA (Figure 5), consistent with previous work.[29] This example further proves the applicability of the magnetic particle based particle counting immunoassays for determination of other DNA modifications in genomic DNA.

The developed particle counting immunoassay can detect trace BPDE−dG adducts as low as 180 fM in the presence of about 1 billion times more undamaged nucleotides in genomic DNA and in cultured cells exposed to a low dose of *anti*-BPDE (1 nM), proving its high sensitivity and specificity. It is evident that the high affinity and the negligible cross-reaction of mAb 8E11 may contribute to the high specificity of the method. On the other hand, the low nonspecific absorption of normal DNA on negatively charged magnetic particles may also make a contribution to the method specificity because of the electrostatic exclusion of the same charge property. The slightly negative charge of the magnetic particles may result from their surface modification by silane.

More importantly, the development of particle counting technology makes a significant contribution to the sensitivity and specificity of the method. Due to its positive charge property, the staining OliGreen dyes can be nonspecifically adsorbed on the negatively charged surface of the cover glass and magnetic particles. Although it has a negligible intrinsic fluorescence, the

surface-adsorbed OliGreen can induce interfering background fluorescence (Figure 6a). Fortunately, this background fluorescence of absorbed OliGreen dye can be effectively eliminated by our prequenching protocol developed in this work. It was found that the fluorescence of absorbed OliGreen dye could be easily quenched by exposure for only a short time (less than 30 s) under the irradiation of blue light (450−490 nm) (Osram, Germany) at a relatively low power (10%), while this prequenching procedure has little influence on the fluorescent response of the OliGreen−ssDNA complexes (Figure 6a). After being prequenched under blue light for only 10 s, the green background fluorescence on the cover glass was reduced by about 60% and the ratio of fluorescent intensity between the fluorescent dots and background (S/N) was enhanced at least 3-fold (Figure 6a). The prequenching can also reduce the fluorescence of OliGreen dye absorbed on magnetic particles, which may cause false-positive results. After 30 s of prequenching, the number of fluorescent dots was sharply reduced by about 86.3% for the DNA sample without BPDE adducts, while the number was only reduced by about 29.3% for the DNA sample with BPDE adducts (Figure 6b). Therefore, excess OliGreen dye uncomplexed with ssDNA is more favorable to be quenched, and this has been exploited to improve the signal-to-noise ratio for fluorescent imaging in this work.

The particle counting technology can also provide an advantage over traditional microplate based immunoassays. By means of setting an appropriate fluorescent dot counting threshold, the specific binding of the OliGreen−ssDNA complex can be distinguished from the nonspecific adsorption of OliGreen on particle surfaces due to the weak fluorescence of the latter. It is also possible to distinguish the specific binding of the OliGreen−ssDNA complex from the nonspecific adsorption of OliGreen-stained ssDNA. Regarding the property of nonspecific adsorption, it is expected that the nonspecific adsorption of ssDNA on a negatively charged particle surface may have a much weaker affinity, leading to less ssDNA molecules bound to a particle and emitting a weaker fluorescence signal. In traditional microplate based immunoassays, the total fluorescence intensity is estimated rather than determined by counting particle by particle for quantification. In such a format, the specific and nonspecific binding can contribute to the total fluorescence signal together. Therefore, the nonspecific adsorption of a fluorescent dye on a solid phase cannot be differentiated from the specific binding of the fluorescent dye to target molecules in traditional microplate based immunoassays.

## CONCLUSIONS

In summary, we demonstrate a particle counting technology for the immunodetection of DNA modifications with high sensitivity and specificity. The innovated approach design takes advantage of the signal amplification from the multiple binding of a specific fluorescent dye to ssDNA molecules and magnetic separation. The multiple labeling of captured DNA greatly brightens the particle, allowing for particle counting and greatly enhancing the detection sensitivity. The particle counting technology probably provides the capacity of differentiating the specific binding from nonspecific interactions and the possibility to achieve high specificity. The technology is applicable to the detection of BPDE−DNA adducts and global DNA methylation, showing its wide applications in DNA modification analysis. The applicability of the technology from short oligonucleotides to genomic DNA further supports its promising applications.

## ACKNOWLEDGMENT

This work was supported by grants from the National Basic Research Program of China (09CB421605 and 2010CB933502 and) and the National Natural Science Foundation of China (21077129, 20877091, 20805057, and 20890112).

## SUPPORTING INFORMATION AVAILABLE

Additional information as noted in the text. This material is available free of charge via the Internet at http://pubs.acs.org.

Received for review September 10, 2010. Accepted October 26, 2010.

AC102416F