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Immunomagnetic Diffractometry for Detection of Diagnostic Serum Markers

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Abstract: We describe an integrated approach for detection of diagnostic markers using in situ assembled optical diffraction gratings in combination with immunomagnetic capture. Folate receptor (FR), a serum protein indicative of various cancers, was chosen as a model system to demonstrate the potential of the method. Magnetic beads coupled to FR antibody were used to capture FR from serum. The FR-bound magnetic beads self-assembled onto microcontact-printed folate-coupled BSA (F-BSA) patterns to form diffraction gratings which served to detect FR by measuring the diffraction intensities caused by laser illumination. The FR-containing beads, upon binding to the F-BSA surface, served as intrinsic signal enhancement agents, circumventing the need for additional enzymatic signal amplification or fluorescent labeling steps. With this approach, a detection sensitivity of 700 fM (20 pg/mL) was achieved. The potential use of this approach in clinical diagnostics was demonstrated by measuring FR concentration in blood samples obtained from cancer patients.

Introduction

Early detection of molecular biomarkers in serum is an extremely important step toward successful disease diagnosis. Serum markers can reveal significant information about the onset and progression of many diseases ranging from heart attacks to malignancies.^{1,2} For example, the markers myoglobin (MG), cardiac troponin I (cTnI), and C-reactive protein are known as strong indicators of heart problems,^{2,3} whereas serum levels of cancer antigen 125 (CA125) and prostate-specific membrane antigen (PSMA) are known to be correlated to ovarian cancer and prostate cancer, respectively.^{4,5} Methods currently available for detection of disease biomarkers include fluorescence immunoassays,⁶ enzyme-linked immunosorbent assays (ELISAs),⁷ PCR approaches,⁸ biobarcode assays,⁹ quartz crystal microbal-

ance analyses,¹⁰ electrochemical methods,^{11,12} microcantilever detection,¹³ and nanoparticle-based biosensors.¹⁴ Despite the diversity of available detection platforms, important challenges still remain in minimizing sensor size, reducing detection time, eliminating target labeling, minimizing signal amplification steps, and developing simple and inexpensive fabrication protocols.¹⁵

Recently, increased attention has been paid to the development and application of separation techniques that employ small magnetic beads to capture a desired analyte from complex biological samples.¹⁶ Magnetic beads have been employed for DNA isolation (followed by PCR amplification),¹⁷ in genomics,¹⁸ and in affinity capture and purification of proteins and peptides.¹⁹ Also, magnetic beads coupled to Ni-NTA (nitrilotriacetic acid) ligands have been used in the magnetocapture,

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purification, and detection of histidine-tagged proteins.²⁰ Although magnetic bead-based strategies have greatly improved analyte capture, their potential for biosensing applications is limited by the labor and time required to quantitate the captured biomarker. To this end, combining a one-step magnetic bead-based capture scheme with a fluorescence or radio-label-free detection scheme would constitute a significant advance.

Biomolecular detection using optical diffraction gratings is a versatile fluorescence or radio-label-free method,²¹ and in comparison with most detection schemes described above, it has relatively straightforward experimental instrumentation.^{22–26} However, the microfabrication of diffraction gratings is an additional step that can introduce additional cost and labor issues to its practical applicability. Also most of the available diffraction grating-based detection methods require additional signal amplification steps (after the desired analyte is captured on a solid surface), such as the sequential use of nanoparticle-conjugated antibodies,²⁷ and/or enzymes to improve the sensitivity of detection, leading to time-consuming, multistep procedures.²⁸

Herein we describe the development of an integrated detection system that combines immunomagnetic capture and optical diffraction for rapid detection of disease biomarkers. In this detection system, the magnetic beads function both as regular immunomagnetic capture agents and as motifs for the in situ assembly of optical diffraction gratings. The magnetic beads, because of their large size compared to the target molecules, significantly enhance the signal intensity upon diffraction grating formation, thereby greatly lowering analyte detection limits. The method is rapid, simple, inexpensive, and devoid of complex microfabrication procedures and can detect extremely small clinically significant target concentrations.

We applied the method to detection of folate receptor (FR), also known as the folate binding protein (FBP): a protein that is overexpressed on the surfaces of malignant cells which eventually enters into the blood, thus serving as a potential tumor biomarker.^{29,30} Immunomagnetic diffractometry could detect as low as 700 fM (about 20 pg/mL) FR, without requiring any fluorescent or radio labels or enzymatic signal amplification steps. The method is generic and can be readily applied to many other molecular targets.

Results and Discussion

It is known that several types of cancers overexpress specific proteins on the tumor cell surface which enter the blood stream and serve as clinical biomarkers for neoplastic lesions.¹ The FR has been shown to be overexpressed in many cancer cell types, and increased levels have been found in the sera of cancer

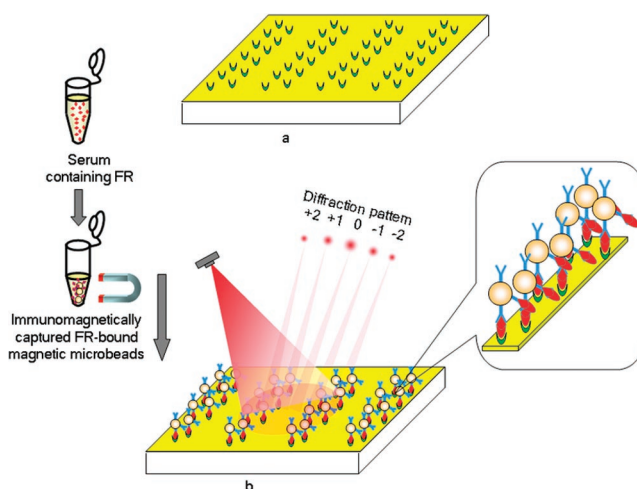


Figure 1. Schematic of immunomagnetic diffractometry and its application to detection of FR. The FR present in the serum solution is immunomagnetically captured by magnetic beads. (a) Microcontact-printed folate-BSA (F-BSA) patterns. (b) Self-assembled FR beads forming diffraction gratings on F-BSA micropatterns. Illumination with a laser yields a characteristic diffraction pattern whose modal intensities depend on the density of the attached beads.

patients.³⁰ Given the emerging interest in folate-based diagnostics, we applied immunomagnetic diffractometry to detection of FR. The optical diffraction-based FR detection study that we describe here includes (a) immunomagnetic capture of FR from serum, (b) in situ assembly of optical diffraction gratings, and (c) optical diffraction intensity measurements (Figure 1).

Immunomagnetic Capture of FR from Serum. The FR antibody-coupled magnetic beads (Ab-beads) were used for the immunomagnetic separation of FR from serum. Ab-beads were prepared by reacting *N*-hydroxysuccinimide (NHS)-activated magnetic beads (968 nm mean diameter) with FR antibody solution. The unreacted NHS groups present on the magnetic beads were then quenched by addition of ethanolamine.

FR present in 10% fetal calf serum in PBS was immunomagnetically captured on Ab-beads. Aliquots of Ab-beads were separately incubated with increasing concentrations of FR solutions (700 fM to 11 nM) for 10 min while maintaining a constant number of Ab-beads (1.8×10^8 beads), followed by rinsing with PBS in a magnetic separator to remove unbound FR and obtain pure FR-bound beads (FR-beads).

In Situ Assembly of Optical Diffraction Gratings. FR is known to specifically bind to folic acid in a 1:1 ratio with strong affinity ($K_d = 10^{-10}$ M).¹⁰ Accordingly, we microcontact printed patterns of folate-coupled bovine serum albumin (F-BSA) onto gold-coated glass slides (gold chip) as an FR-recognition element. F-BSA bound strongly to the gold surface, providing a robust micropattern upon printing with an elastomeric stamp containing 15 μ m wide alternating linear patterns.^{10,31} Importantly, F-BSA was deposited only in the direct contact areas on the gold chip, rendering the micropatterns as well-defined templates for self-assembly of FR-beads.

A suspension of FR-beads in PBS was transferred onto the gold chip containing F-BSA micropatterns for the in situ assembly of an optical diffraction grating. FR-beads were observed to specifically attach to the F-BSA micropatterns,

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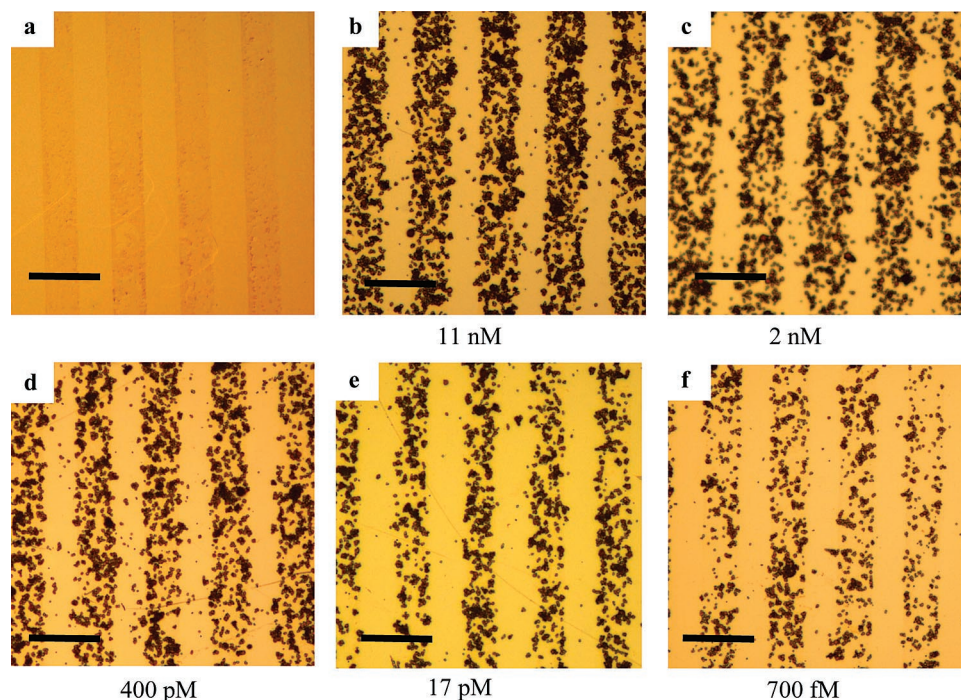


Figure 2. Optical micrographs of in situ assembled diffraction gratings. (a) Micropattern of F-BSA stamped onto a gold chip. (b–f) Diffraction gratings formed by FR-beads following their self-assembly on F-BSA-printed patterns. The packing density of FR-beads decreases with decreasing concentrations of FR from 11 nM to 0.7 pM. Scale bar = 30 μm .

while unbound FR-beads were removed by rinsing with PBS. Finally, the gold chip was rinsed with nanopure water to remove buffer salts and dried under a gentle stream of nitrogen. The FR-bead micropatterns were characterized by optical microscopy (Figure 2). These well-defined and robust magnetic bead patterns functioned as optical diffraction gratings and were used for diffraction intensity measurements.

To optimize incubation times, we performed time-dependent experiments to assess the assembly of FR-beads on F-BSA micropatterns. Freshly microcontact-printed gold chips bearing F-BSA micropatterns were incubated with FR-beads and examined under an optical microscope at 5 min intervals over a 30 min period. Subsequent optical microscopic examination and diffraction analysis revealed that a 10 min exposure to the FR-bead suspension resulted in a diffraction grating with minimum nonspecific binding of the beads in the interpattern region (neat gold surface). Longer incubation times (> 10 min) resulted in increased nonspecific binding of the magnetic beads in the interpattern region, forming an irregular grating with a weak diffraction signal intensity.

Optical Diffraction Measurements. Diffraction-based sensing is a simple and powerful technique that can be used to observe biomolecular interactions. A diffraction grating is a periodic pattern of uniformly spaced lines. When a laser beam illuminates a diffraction grating, a diffraction image of evenly spaced bright spots of varying intensities is produced.³² In our experiments, microcontact-printed F-BSA patterns functioned as biomolecular ligands, and the binding of FR-bound magnetic beads on these patterns resulted in an in situ diffraction grating, whereby the presence of beads causes comparative changes in the modal intensity.³³

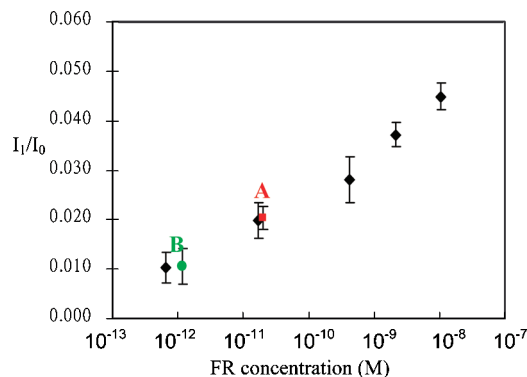


Figure 3. Variation of the normalized diffraction intensity (I_1/I_0) with the FR concentration. The diffraction intensity increases with the FR concentration (0.7 pM to 11 nM). Diluted blood samples obtained from cancer patients: Sample A (red square) corresponds to an approximate FR concentration of 20 pM, while that of sample B (green circle) is ~ 1 pM (error bars correspond to three measurements).

Illuminating the surface with a laser beam (He-Ne, 632 nm) produced a row of diffraction modes with varying intensities due to the interference of laser light beams reflecting from the beads and bare gold surface (Figure 1). The diffraction signal intensities depend upon the packing density as well as the diameter of the beads used. Monitoring the intensities of the diffraction modes enabled the measurement of binding. (The schematic of the diffractometry setup and size information about the beads is presented in the Supporting Information; see Figure S1.) The intensity of the diffraction modes varied with the packing density of the micropatterns. The number of magnetic beads self-assembled onto the micropatterns increased with increasing concentration of FR, leading to more densely formed micropatterns (Figure 2). This in turn resulted in an increase in intensity of the first diffraction mode with respect to the zeroth one.

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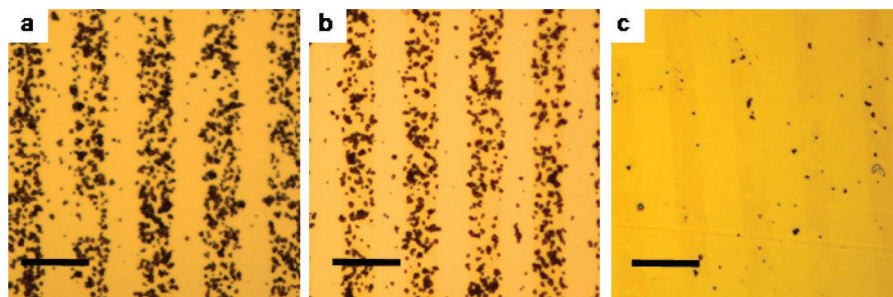


Figure 4. Analysis of diluted blood samples obtained from cancer patients. (a, b) In situ assembled diffraction gratings using immunomagnetically separated FR-beads following their incubation in the sera of two unrelated cancer patients (samples A and B, respectively). (c) A diffraction grating was not formed with immunomagnetically separated FR-beads incubated in blood serum of a healthy person (control experiment). Scale bar = 30 μm .

We studied the intensity ratio of the first and the zeroth modes, i.e., I_1/I_0 , versus the concentration of FR (700 fM to 11 nM). Figure 3 shows that the binding of beads increased the first diffraction mode signal with respect to the zeroth mode, resulting in an increase in the diffraction efficiency. Normalizing the modal intensities in this way also served to suppress the effects of possible drifts in incident laser intensity and small variations from sample to sample.³³

To examine the potential of this biosensor in clinical diagnostics, we tested blood samples (A and B) from two cancer patients diagnosed with different types of cancer. For appropriate comparison with the serum experiments described above, the patients' sera were diluted 10-fold in PBS. FR was immunomagnetically captured from the blood samples using the Ab-beads. The FR-beads were spotted on a gold chip containing the usual F-BSA micropattern. Once again, the FR-beads self-assembled on F-BSA micropatterns, forming diffraction gratings (Figure 4a,b). The corresponding concentrations for samples A and B were approximated (by fitting a curve to the previously described serum data) to be about 20 and 1 pM, respectively (Figure 3).

The FR concentration in the *nondiluted* blood sample A determined independently by ELISA was 300 pM. Because of the very low levels of FR in sample B, we could not determine the FR concentration with ELISA. As a control, we tested the blood sample obtained from a healthy individual following the same experimental procedure. In this case, FR-beads did not self-assemble on the F-BSA micropatterns in significant amounts, and no diffraction grating formation was observed (Figure 4c). These results demonstrate the potential of the method as an ultrasensitive and rapid biosensor for clinical diagnostics.

In all experiments, the detection area of the micropattern scanned was 0.0225 mm², comprising five bead-containing stripes, each of 15 μm width and 150 μm length. A characteristic diffraction pattern was obtained only when the FR-beads self-assembled onto the F-BSA patterns. Neat F-BSA patterns, in the absence of FR-beads, did not produce a diffraction signal. The approximate number of magnetic beads present per detection area varied between 5900 (for 11 nM) and 3100 (for a 700 fM concentration of FR), suggesting that the number of beads bound to the F-BSA micropattern varied monotonically with the FR concentration. The number of FR molecules captured by a single Ab-bead (for an 11 nM FR concentration) was approximated by micro-BCA assay experiments³⁴ to be approximately 150 (see the Supporting Information for assay details). Micro-BCA assay was not sensitive enough to reveal

the number of FR molecules captured for FR solutions at concentrations below 10 nM. For these concentrations, the number of FR molecules captured by each Ab-bead was roughly estimated by dividing the number of FR molecules theoretically present in an FR solution of a given concentration by the magnetic bead number. The number of FR molecules captured per bead decreased from \sim 150 molecules (at 11 nM) to \sim 1 molecule (at 0.7 pM). Hence, with this method we could detect as few as 3100 molecules per detection area. The number of FR molecules present on the bead surface corresponds to the number of available binding sites for the folate ligands, and each FR molecule binds to the folate ligand in 1:1 stoichiometry. Since the FR molecules are randomly distributed on the bead surface, not all the FR molecules on a bead will be participating in binding and only a few FR molecules contribute to the binding event. Hence, the number of FR molecules present per bead is only an approximation, and the beads that are eventually captured on the substrate from the total bead population may possibly contain a larger number of FR molecules.

Immunomagnetic diffractometry, in spite of its relative simplicity, has excellent detection limits. In a recent study, surface plasmon resonance has been shown to detect as low as 130 ng/mL FR.³⁵ The microarray-based ELISA can detect as little as 13.4 ng/mL IgG monoclonal antibody.³⁶ With a quartz crystal microbalance (QCM) biosensor using gold nanoparticles as a signal amplification element, a detection limit of 1.5 ng of FR/mL has been achieved, while an enzyme-amplified diffraction-based immunoassay has been reported to detect as low as 50 pg/mL antidigoxin.^{10,28} Most of the presently available detection methods use time-consuming labeling or sequential signal amplification procedures (after capturing target molecules on a solid surface) to detect molecules in low quantities. Immunomagnetic diffractometry was able to detect as low as 700 fM FR (20 pg/mL) without requiring any sequential labeling or amplification procedures.

Evidence for the Specific Assembly of FR-Beads on F-BSA Micropatterns. To demonstrate the requirement of folate ligands for specific binding of FR-beads, we microcontact-printed BSA (lacking conjugated folate) on a gold chip. Onto this chip was transferred an aliquot of FR-beads. Optical microscopic examination of the chip revealed that the FR-beads did not bind significantly to the underivatized BSA, thus

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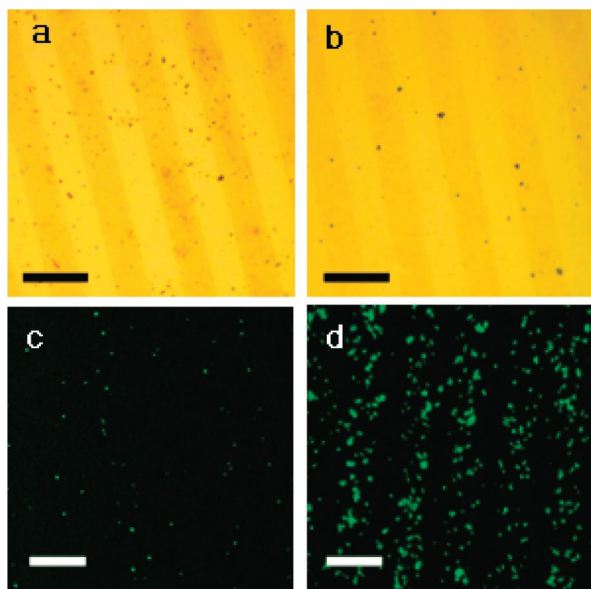


Figure 5. Control experiments demonstrating the requirement for FR for in situ formation of diffraction gratings. (a) FR-beads did not bind to underivatized BSA micropatterns in significant numbers. (b) Antibody-coupled beads did not bind significantly to the F-BSA micropatterns in the absence of FR. Folate competition experiments demonstrating the specific binding of FR to folate. (c) FR-beads saturated with folate-FITC did not self-assemble significantly on F-BSA patterns to form diffraction gratings. (d) FR-beads partially saturated with folate-FITC did self-assemble on F-BSA patterns. Scale bar = 30 μm .

demonstrating the requirement of folate ligands for in situ assembly of diffraction gratings (Figure 5a).

To test the specific requirement for capture of FR, we incubated a gold chip containing F-BSA micropatterns with Ab-beads devoid of FR. As expected, Ab-beads did not bind significantly to the F-BSA micropatterns in the absence of FR, demonstrating the requirement of FR (Figure 5b).

To further confirm the specific binding of FR-beads to folate ligands, we conducted competition experiments using fluorescently labeled folate (folate-FITC). A suspension of Ab-beads was treated with an FR solution (50 nM in 10% fetal calf serum). The FR-beads were then treated with folate-FITC solution in PBS (200 nM) to saturate folate binding sites. The folate-FITC-saturated FR-beads were then transferred onto F-BSA micropatterns. The beads did not bind in significant numbers to the F-BSA micropatterns, confirming the requirement of FR with free folate binding sites (Figure 5c). A similar experiment was performed with 25 nM folate-FITC to obtain partially saturated FR-beads. These partially saturated FR-beads did self-assemble on F-BSA micropatterns to form loosely packed gratings as they had some folate binding sites available on them (Figure 5d).

Conclusions

In this paper we presented a magnetic bead-based optical diffraction strategy, i.e., immunomagnetic diffractometry that is capable of detecting FR at concentrations as low as 20 pg/mL (700 fM) without postcapture labeling (with fluorescent or radioactive labels) or multiple signal enhancement steps. We also demonstrated its potential in clinical diagnostics by testing blood samples obtained from patients with different types of cancer.

Self-assembly of analyte-containing microbeads in an alternating line pattern formed a diffraction grating which in turn affected the intensity of diffracted light and enabled detection of biomolecular targets in femtomolar-level concentrations. Distinct and measurable diffraction patterns were obtained only when the FR-beads self-assembled on the F-BSA patterns. Since biological molecules are generally a few nanometers in size, their sole binding (without any solid particles) to a solid surface results in relatively weak diffraction patterns. The use of micrometer-sized magnetic beads for molecular separation inherently circumvented this drawback. Hence, the magnetic beads played a dual role: (a) as agents of immunomagnetic capture from complex mixtures and (b) as in situ generated optical diffraction gratings allowing direct, fluorescence or radio-label-free detection.

Immunomagnetic diffractometry is a method that inherently accommodates both biomolecular separation and detection. It is robust, rapid (both to set up and to operate), inexpensive, and prone to miniaturization. Furthermore, it allows the primary target capture to occur in solution (not on a surface), enabling mass transport in three dimensions and circumventing reduced reaction rates that result from capturing the primary analyte on a two-dimensional surface.^{37,38} Finally, the method is generic, and hence, the same principles presented here should apply for detection of many other disease markers present in various body fluids. Due to its simplicity and high sensitivity, we expect this method to be extremely useful both in research laboratories and in development of devices for point-of-care diagnostics.

Materials and Methods

Materials. Gold-coated glass slides (50 nm thick gold layer with 5 nm thick chromium as an intermediate layer between glass and gold) were purchased from Asylum Research and were cut into 1.5 cm \times 1 cm chips. These gold chips were cleaned with ethanol and dried prior to use. PBS was purchased from GIBCO. NHS-activated magnetic beads (968 nm mean diameter) were obtained from Chemagen. FR was purchased from Scripps Research Institute. The FR antibody was obtained from Endocyte Inc. BSA and folic acid were purchased from Sigma. Fetal calf serum was obtained from Atlantic Biologicals. The micro-BCA protein assay reagent kit was purchased from Pierce Biotechnology. Blood samples from cancer patients were obtained under the auspices of an IRB-approved protocol at the Mayo Clinic, Rochester, MN. Optical micrographs were obtained using a Nikon (Eclipse 80i) microscope connected to a CCD camera. Laser scanning confocal fluorescence images were acquired using an MRC-1024 UV instrument (Bio Rad, Hemel Hempstead, England) on a Diaphot 300 (Nikon, Tokyo, Japan) inverted microscope using a 20 \times 0.75 NA lens. The 488 nm wavelength of the krypton-argon laser (Ion Laser Technology) was used to excite the green fluorochrome. Green fluorescence was collected with a 525/35 band-pass filter.

Methods. 1. Diffractometry Setup. The schematic of the instrument setup is presented in Supporting Information Figure S1. A laser beam (He-Ne laser, Newport R-30991, 633 nm, 5 mW) was passed through a beam splitter (Thorlabs, BS016) and a convex lens (focal length 60 mm) to obtain an incident beam diameter of \sim 150 μm on the gold chip surface. The reflected beam was passed through a beam expander (Edmund Optics) to separate the modes of the diffraction pattern and focus them onto the photodiode. A silicon photodiode (12 V reverse bias, Thorlabs DET110) affixed with an adjustable aperture (Thorlabs SM1D12) and a band-pass filter (Thorlabs FL632.8-10) was fixed on

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a translational stage (Thorlabs MT3) for fine adjustments. The photodiode was connected to a variable resistor (10 k Ω , Thorlabs) to convert the output current into voltage, which was in turn fed into a low-pass amplifier (Stanford Research SR640), and the data were processed by a computer with a National Instruments Lab View interface.

2. Preparation of FR Antibody-Coupled Magnetic Beads (Ab-Beads). To the FR antibody solution (1 mg/mL in PBS, 7.2 pH) were added NHS-activated magnetic beads (1.8×10^{10} beads/mL, mean diameter of 968 nm, obtained from Chemagen), and the suspension was shaken for 2 h. At the end of this period, the suspension was placed in a magnetic separator, washed with PBS three times, and resuspended in PBS. The unreacted NHS groups present on the magnetic beads were quenched by treatment with ethanolamine (0.5 mM solution in PBS) followed by rinsing three times with PBS and resuspension in 1 mL of PBS. The Ab-bead suspension was stored at 4 °C.

3. Immunomagnetic Capture of FR. A series of concentrations (0.7 pM to 11 nM) of FR dissolved in serum solution (10% fetal calf serum in PBS) were used in the experiments. A suspension of Ab-beads (1.8×10^8 beads in 10 μ L of PBS) was transferred into a 1 mL Eppendorf tube, which was placed in a magnetic separator, and the liquid was completely removed. To the neat Ab-beads was added 50 μ L of FR solution in serum, and the suspension was shaken for 15 min. At the end of this period, the Eppendorf tube containing the magnetic bead suspension was again placed in a magnetic separator, washed with PBS (3 \times 50 μ L) to remove unbound FR, and resuspended in 50 μ L of PBS. The FR-beads thus obtained were used for the in situ assembly of diffraction gratings on F-BSA micropatterns.

4. Construction of PDMS Stamps. PDMS stamps were prepared with a master mold having 15 μ m alternating stripes. The negative mold was designed to give the resulting stamp 15 μ m wide stripes. Silicone elastomer base (SYLGARD 184 silicone elastomer kit, Dow Corning Corp., Midland, MI) and curing agent were mixed in a 10:1 ratio, degassed for 1 h, and then poured into the master. Curing was carried out at 60 °C overnight. The cured PDMS stamp was carefully peeled off the mold after they were cooled to room temperature. The stamp was thoroughly rinsed with nanopure water, dried with nitrogen gas, and stored in a vacuum desiccator prior to use.

5. Microcontact Printing of F-BSA. The F-BSA (1 mg/mL in PBS, pH 7.2) solution was applied onto the PDMS stamp surface with a Q-tip cotton swab and left to stand for 2 min. F-BSA was prepared according to a published procedure.¹⁰ Excess solution was removed by drying the stamp under a gentle stream of nitrogen gas. The stamp was brought into contact with a gold chip and gently pressed to make a good contact between both surfaces. The stamp was removed after 2 min, and the gold chip was rinsed with PBS to remove any unbound F-BSA.

6. Self-Assembly of FR-Beads on F-BSA Micropatterns. A suspension of FR-beads (1.8×10^8 beads in 50 μ L of PBS) was

transferred onto a gold chip containing F-BSA micropatterns, and both were incubated for 10 min. At the end of this period, the gold chip was rinsed with PBS and with nanopure water (to remove buffer salts) and dried under a gentle stream of nitrogen.

7. Exposure of FR-Beads to Neat BSA Micropatterns. The neat BSA micropatterns were prepared by following exactly the same procedure as that for F-BSA micropattern preparation, substituting BSA for F-BSA. The FR-beads were exposed to neat BSA micropatterns following exactly the same procedure as described in "Self-Assembly of FR-Beads on F-BSA Micropatterns".

8. Exposure of Ab-Beads to F-BSA Micropatterns. Ab-beads were transferred onto the gold chip containing F-BSA micropatterns (1.8×10^8 beads suspended in 50 μ L of PBS), and both were incubated for 10 min. At the end of this period, the chip was rinsed with PBS and nanopure water and dried with nitrogen.

9. Folate Competition Experiments. An FR solution (50 μ L, 50 nM) in 10% fetal bovine serum was added to Ab-beads (same quantity and preparation as described before) and the suspension shaken for 15 min. At the end of this period, the FR-beads were rinsed with PBS (3 \times 50 μ L) in a magnetic separator and the liquid was completely removed. To the neat FR-beads was added folate-FITC solution in PBS (50 μ L, 200 nM) and the suspension shaken for 15 min. At the end of this period the magnetic beads were rinsed with PBS (3 \times 50 μ L) in a magnetic separator and resuspended in 50 μ L of PBS. Another sample was prepared by following exactly the same procedure but using 25 nM folate-FITC. The folate-FITC-treated FR-beads were separately transferred onto two gold chips containing F-BSA micropatterns, and both were incubated for 10 min. Then the chips were rinsed with PBS and nanopure water and dried with nitrogen gas.

10. Experiments with Blood Samples of Cancer Patients. The blood samples were obtained from Mayo Clinic. Sample A was from a patient that was diagnosed with stage III, grade III serous ovarian cancer, and sample B was from a patient with stage IV leiomyosarcoma of the uterus. The sera were separated from the blood samples by centrifugation and diluted 10-fold for direct comparison with experiments which were performed by adding target molecules to 10% sera. The experiments involving immunomagnetic separation of FR and the assembly of FR-beads onto F-BSA patterns were performed by following exactly the same procedures as described earlier.

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Supporting Information Available: Schematic of the diffractometry setup (Figure S1) and micro-BCA assay experimental procedure. This material is available free of charge via the Internet at <http://pubs.asc.org>.

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