

Spinning-disk self-referencing interferometry of antigen-antibody recognition

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A gold ridge microstructure fabricated to a height of $\lambda/8$ on a high-reflectivity substrate behaves as a wave-front-splitting self-referencing interferometer in phase quadrature when illuminated by a Gaussian laser beam and observed in the far field along the optic axis. When immuno-gammaglobulin (IgG) antibodies are selectively immobilized on the gold microstructure, they recognize and bind to a specific antigen, which shifts the relative optical phase of the interferometer and modifies the far-field diffracted intensity. We detect bound antigen interferometrically on spinning disks at a sampling rate of 100 kHz and verify the interferometric nature of the signal by using two quadratures of opposite sign to rule out effects of dynamic light scattering. Strong molecular recognition is demonstrated by the absence of binding to nontarget molecules but strong signal change in response to a specific antigen. This BioCD has the potential to be applied as a spinning-disk interferometric immunoassay and biosensor. © 2004 Optical Society of America
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Microarray sensors have become valuable tools in gene-sequencing efforts carried out in industry and in academia.¹⁻⁴ Sensors have been developed on a multitude of different platforms with a variety of approaches,⁵ many of them optical.^{6,7} The readout mechanism of a majority of the sensors, especially microarray sensors, is based on the fluorescence of tagged molecules. Although fluorescence is sensitive and capable of single-molecule detection, it relies on emission from fluorophores with typically low conversion efficiency and a tendency to photobleach, both of which result in low detected photon fluxes and long data acquisition times. Direct detection with interferometry⁸⁻¹² operates with much-higher photon fluxes, but most interferometric implementations do not have the massive parallelism that is needed for high-multiplicity multianalyte assays. Here we propose and demonstrate an interferometric detection scheme that has a high data-sampling rate and the high sensitivity offered by interferometry with the potential for high-multiplicity multianalyte assays.

We have implemented interferometry in a platform similar to the so-called optical compact disk (CD) that supports more than a billion separate interferometric elements that are embossed on a disk a few inches in diameter and spun at high speeds.¹³ The CD elements are self-referencing. Self-referencing interferometers generate the reference wave locally with respect to the signal wave so that the reference and signal waves experience common aberrations and path-length changes and thus maintain constant relative phase without the need for active stabilization of different light paths. To make microfabricated self-referencing interferometers operate as a biosensing platform, we adjusted them to operate in interferometric quadrature, providing linear (analog) sensitivity rather than digi-

tal. Biological macromolecules such as antibodies, antigens, or DNA can be immobilized on the microinterferometric elements. These subsequently act as receptors for specific target analytes, modifying the phase of the interferometric element, which translates into a modification of the detected far-field diffraction intensity. This so-called BioCD can be used as an immunosensor where a solution can be screened for the presence of antigens specific to the antibodies immobilized on the interferometric elements.

A dielectric mirror with a center operating wavelength of 633 nm was chosen as the substrate for the fabrication of our BioCD. We created the microinterferometric elements by evaporating a radial pattern of 1024 gold lines, 20 μm wide, arranged as radial spokes, as shown in Fig. 1(a). The advantage

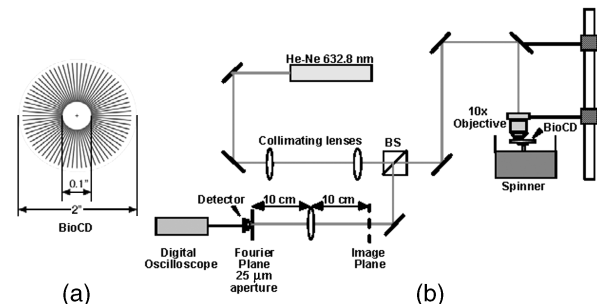


Fig. 1. (a) Schematic of the gold spokes evaporated on a 2-in. (5.08-cm) dielectric mirror substrate. There are 1024 spokes on a single disk, but all of them are not shown in the diagram for clarity. (b) Schematic of the experimental setup to detect the far-field diffraction from the BioCD. The 25- μm aperture is placed at the Fourier plane formed at the focal plane of the lens. BS, beam splitter.

of using gold interferometric elements rather than pits as on a conventional optical CD is the ease with which macromolecules on the interferometric structures are immobilized by use of an alkanethiol bridge between the gold and the macromolecule. By use of polydimethoxy silane stamps,¹⁴ we immobilize antibodies on the gold pattern within annular regions of the disk. Thus a BioCD can be made to possess different regions with and without antibodies to test for antigen binding in the regions containing the antibodies and to test for nonspecific binding.

Gold for the spokes is evaporated to a thickness of 80 nm, making the phase difference between the gold ridge and the land equal to $\pi/2$, in contrast with the π phase condition in a conventional digital CD. This is a significant difference. Interferometers have a universal response curve with a half-intensity point defined by quadrature when the signal and the reference waves are out of phase by 90° . Maximum linear sensitivity to small optical phase perturbations is achieved by operating the interferometer near quadrature. Shot-noise-limited detection of optical path-length changes down to 10^{-9} wavelengths is achievable under these conditions.

The BioCD is treated with a 10-mM solution of hexadecane thiol in ethanol to deposit a thiol layer on the gold spokes that adsorbs proteins. Polydimethoxy silane stamps create regions with and without antibodies on the BioCD. After immobilizing antibodies in annular regions, the substrate is washed in a potash-buffered saline solution to remove excess antibodies. The substrate is then exposed to antigen solutions that may be specific or nonspecific to the antibodies immobilized on the BioCD. The wash procedure is repeated after exposure to the antigen solution. Fluorescence images obtained with tagged antibodies verify that the macromolecules are immobilized in a well-defined pattern on only the gold spokes with no signal from the substrate. Atomic force microscope images and ellipsometric measurements also indicate immobilization of a monolayer of the antibody on the gold regions of the substrate.

A collimated He-Ne laser beam (633 nm) is focused by a $10\times$ objective to a beam waist of approximately $40\ \mu\text{m}$ onto the gold ridges on the BioCD mounted on a photoresist spinner (Model P6204, SCS). The BioCD is spun at 6000 rpm during operation with a sampling rate of 100,000 samples/s. As shown in Fig. 1(b), a 10-cm focal-length lens is used to perform a Fourier transform of the image of the gold microstrip, after reflection from the BioCD substrate, to obtain the far-field intensity at the detector plane. A photodetector is placed at the Fourier plane to monitor the far-field intensities in conjunction with a $25\text{-}\mu\text{m}$ aperture to reject the diffraction sidelobes. The signal from the photodetector is captured by a digital oscilloscope.

As the laser beam moves across a gold interferometric element in quadrature, the far-field diffraction intensity drops by approximately 50% compared with the intensity from the mirror substrate. This results in the formation of peaks and valleys in the oscilloscope trace, with the valleys representing the interferometric

signal in quadrature. To test the ability of the BioCD to detect the presence of specific antigens in an analyte, we immobilized antimouse immunoglobulin (IgG) on an annular region covering roughly half the area of the substrate. We incubated a second annular region contained fully within the first annular region, with a solution of nonspecific rabbit IgG and later incubated the same region with a solution of specific mouse IgG. The result of this experiment is summarized in Fig. 2. By comparison of the signal collected from regions with and without antimouse IgG, we can see that the immobilization of the antibody lowers the valleys (at quadrature) while leaving the peaks (from the land) unchanged. Immobilization of biomolecules on the gold interferometric elements changes their optical phase, causing a dip in the valleys. The peaks are unaffected in all cases because no antibody is immobilized on the bare substrate. Exposure to a nonspecific antigen, which in this case is rabbit IgG, causes no additional phase change. However, subsequent exposure to the specific antigen, mouse IgG, causes a further drop in the valley signal level compared with the signal from the antibody layer. Thus the detection scheme is specific to the target analyte and is not blocked by previous exposure to the nonspecific antigen.

To verify that the detection scheme is interferometric in nature and not caused by light scattering, we fabricated devices with reversed interferometric response. This was done by increasing the thickness of the gold spokes from 80 to 240 nm ($\lambda/8$ to $3\lambda/8$). We used devices of these two types to detect the binding of mouse IgG, using immobilized antimouse IgG on the sensor. Immobilization of biolayers increases the optical phase of the gold interferometric elements. In the case of $\lambda/8$ devices the intensity of the far-field diffraction decreases with an increase in phase caused by the immobilization of the antibody, as shown in Fig. 3(a). For a $3\lambda/8$ device, shown in Fig. 3(b), this effect is reversed, causing the intensity

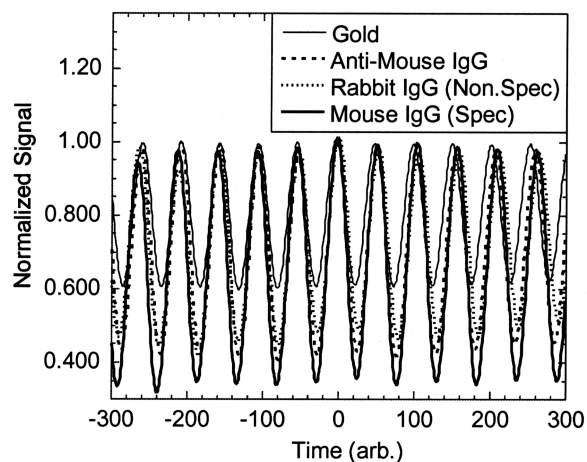


Fig. 2. Detection of mouse IgG; specificity of the detection scheme. The BioCD is printed with antimouse IgG. Incubation with nonspecific rabbit IgG produces no change in the far-field diffraction intensity. However, subsequent incubation with specific mouse IgG produces a drop in the far-field diffraction intensity.

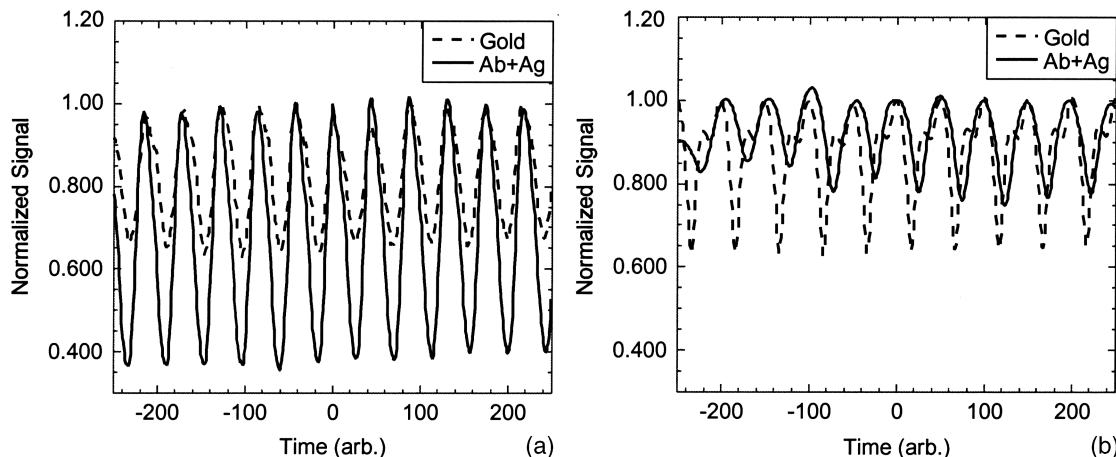


Fig. 3. Verification of the interferometric nature of the detection. (a) Decrease in far-field intensity with an increase in optical phase for a $\lambda/8$ device. (b) Increase in far-field intensity with an increase in optical phase for a $3\lambda/8$ device with reversed interferometric response. The BioCD is printed with antimouse IgG (Ab) and incubated with mouse IgG (Ag).

of the far-field diffraction peak to increase with an increase in phase. As before, immobilization of bilayers affects the valley signal levels but leaves the peaks unchanged. This result proves that the detected signal is interferometric and is detecting the phase modulation caused by the immobilized macromolecules.

In conclusion, we have demonstrated the effectiveness of a fast interferometric approach to biosensing. The number of detected antigen molecules is approximately 10^7 molecules per spoke within the beam waist as indicated by the atomic force microscope results, or a few femtomoles per track. With the current operating signal-to-noise ratio being approximately 100 to 1 the sensitivity of the current system is estimated to be 10^5 molecules per spoke per track. The ultimate detection sensitivity in the shot-noise limit is calculated, based on estimates of detection bandwidth and detected power to be a few molecules per spoke per track. This technique has the distinct advantage of being integrable into a high-speed sensor in an optical disk format delivering high-throughput screening capabilities. This approach would also be useful in applications other than immunoassays, including proteomics, genomics, and drug screening.

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