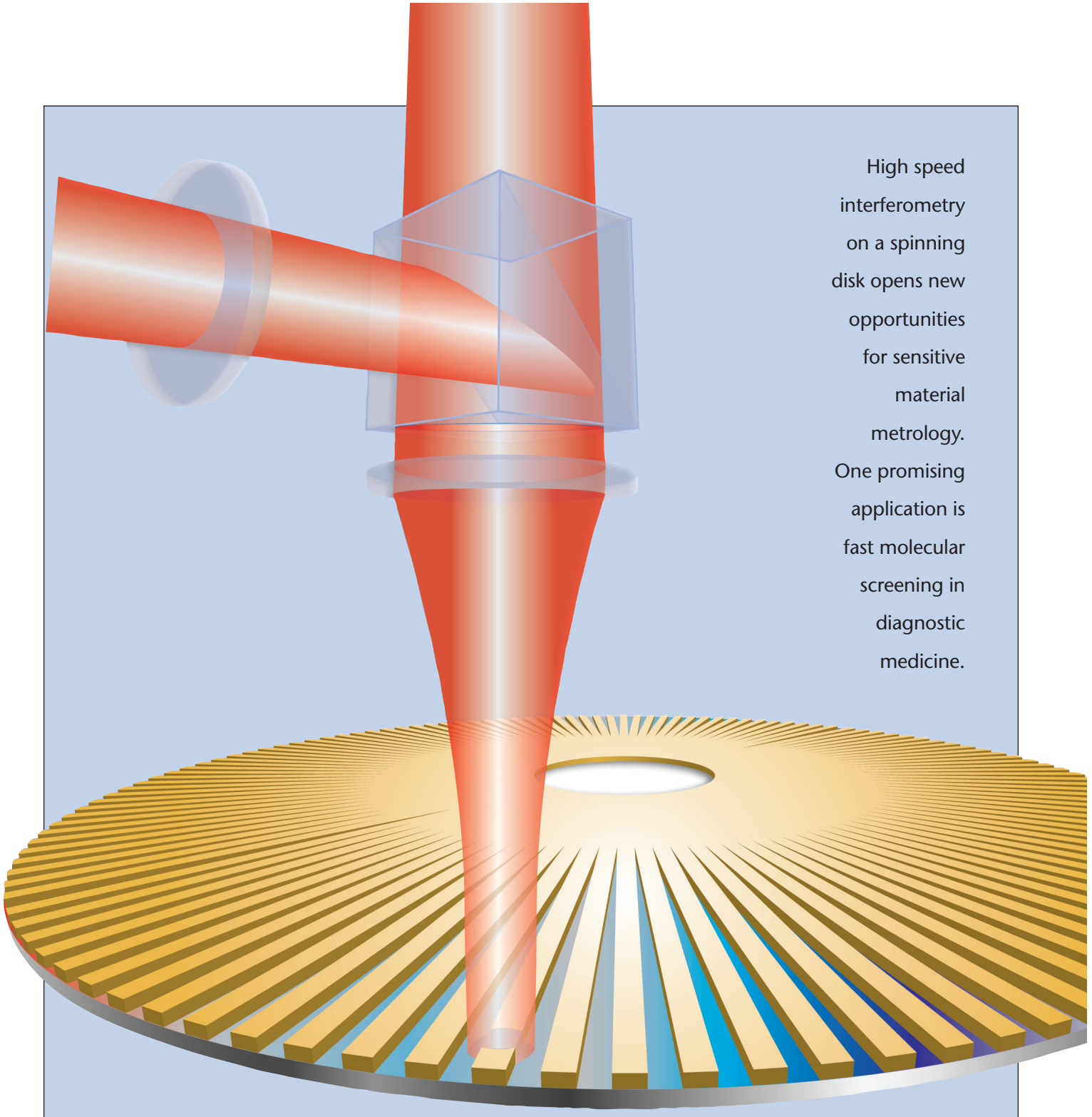

High speed interferometry on a spinning disk opens new opportunities for sensitive material metrology. One promising application is fast molecular screening in diagnostic medicine.

Spinning-Disk Interferometry

The BioCD

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The BioCD is a biological compact disc. It is printed with proteins instead of digital "ones" and "zeros." It reads molecular recognition instead of music. The operation of the BioCD is derived from that of a conventional CD, except for the fact that it operates as an analog sensor rather than as a digital recording medium. While digital CDs are relatively immune to the presence on their surface of fingerprints, the analog BioCD is exquisitely sensitive to submonolayers of immobilized protein. The BioCD is one example of the broader concept of spinning-disk interferometry (SDI), in which intrinsically static measurements (such as molecular recognition) are converted into high-frequency optical modulation.[1] As a biosensor, the BioCD may one day be developed into a fast, cheap and reliable multianalyte assay for clinical medicine and environmental testing.

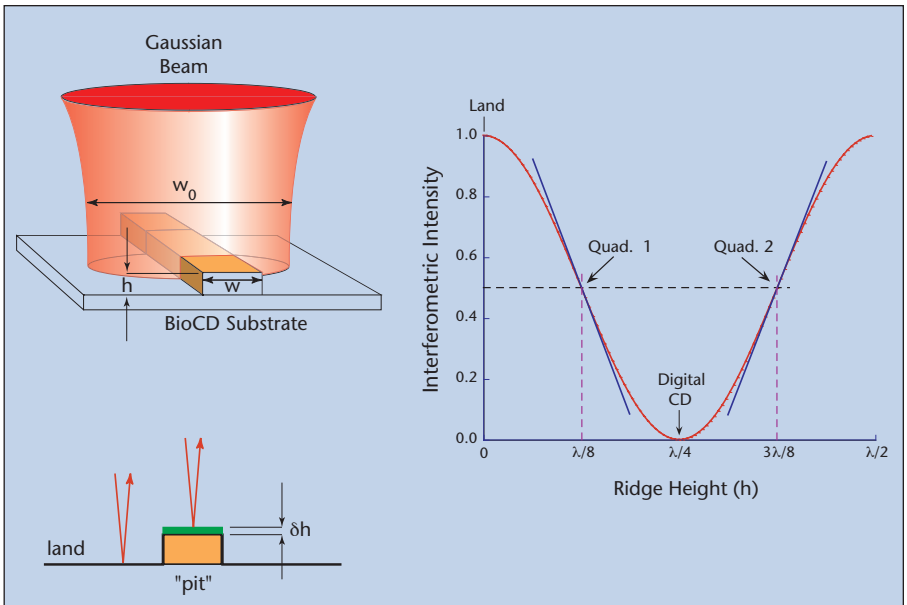
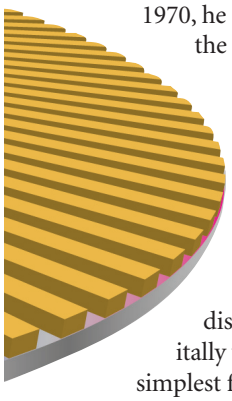


Figure 1. Wave-front-splitting interferometry on a gold microstructured ridge. The far-field intensity along the optic axis follows a sinusoidal interferometric response as a function of height *h*. Destructive interference occurs for a ridge height of λ/4 (used in digital CDs), while maximum linear sensitivity to an added material thickness δ*h* is achieved for the quadrature conditions at $h = \lambda/8$ and $3\lambda/8$.

Compact disc technology

The digital compact disc was invented in 1969 by Klaas Compaan, a Dutch physicist working for Philips Corporation. In 1970, he and Pete Kramer developed the first glass disc prototype and began using a laser to achieve diffraction-limited spot sizes for the readout.[2] The goal was simple: to replace the musical LP—with its grooves and mechanical (analog) scratches—with a disc that could be recorded digitally with ones and zeros. The simplest format is a disk that reflects either high intensities or low intensities to represent the two states. The low reflectance state is produced by scattering light off a small pit. A CD is simply a collection of tracks of pits recessed from the surface (called the land) that are created by embossing a plastic substrate with a glass master. The pits are interrogated by means of a laser focused from the opposite (unembossed) side of the polycarbonate disk, making the indentations appear as ridges, but the "pit" terminology is still often used.



There are approximately three billion pits, on 22 thousand tracks, on a digital data CD. Each pit acts as an individual wave-front-splitting null interferometer, which can be understood by considering Fig. 1. By carefully selecting the focusing lens, a Gaussian laser beam can be made to straddle the pit (ridge) with half the intensity falling on the ridge and half on the land. If the height of the ridge is λ/4, then the phase of the field reflected from the ridge is π out of phase relative to the field reflected from the land. In the far field, along the optic axis, the two fields interfere destructively, producing a null condition at the detector.

As the laser scans a track of pits, the intensity alternates between full intensity reflected from the land, and zero intensity reflected while straddling a ridge. These are the two states that enable digital recording. From Fig. 1 it is clear why fingerprints have so little effect on digital CDs: small changes in pit depth in the land or null states produce only a small quadratic change in the recorded intensity.

The challenge of the spinning disk is to overcome a hostile mechanical environment: vibrations and wobble make it difficult to extract in reflection any signal with a stable average phase, which would seem to preclude the possibility of performing interferometry. Overcoming this challenge is part of the genius of Compaan's original idea for the CD. By illuminating the pit and land by means of a single spatial optical mode, the wave-front-splitting interferometer is self-referencing: the signal and reference waves travel identical paths through the optical system all the way to the detector. This explains why you can jog and still listen to your Discman while it performs a million null interferometric measurements per second.

Spinning-disk interferometry (SDI)

The condition of quadrature is an important alternative that allows the digital CD to be converted into an analog CD that is maximally sensitive to small phase differences. In Fig. 1, when the ridge height is equal to λ/8, the ridge and land signals have a relative phase of π/2. In this case the intensity response curve has a maximum slope, which produces the largest intensity modulation per change in the

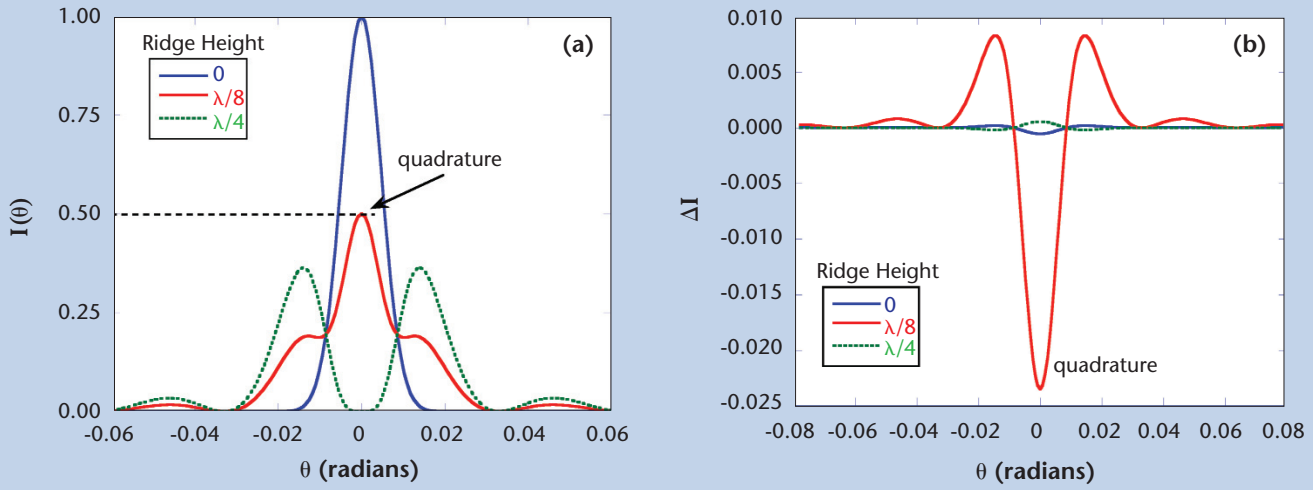


Figure 2. (a) Calculated far-field diffraction for three ridge heights h = 0 (land), λ/8 (quadrature) and λ/4 (null). (b) Relative change in diffraction caused by an extra optical phase $\delta\phi = 4\pi(n\text{-}1)\delta h/\lambda$ for ridge relative to the land. A strong linear response occurs in quadrature.

optical phase of the ridge. In the quadrature condition, the spinning disk takes static structure (surface roughness, index variations, etc.) and turns it into high-frequency phase modulation that can be detected with narrow bandwidth far from 1/f noise. We can apply substantial optical knowledge to the problem since detection of high-frequency phase modulation is common in interferometry.[3]

The spinning disk is a generalized optical modulator that operates in a way similar to a mechanical chopper, except for the fact that it produces small-signal amplitude and/or phase modulation instead of large amplitude modulation, as would be the case, for example, with a fixed-blade chopper wheel. Taken together, small phase modulation and optical phase quadrature make it is possible to operate the device in the linear small-signal limit. This makes it a sensitive analog measurement device of broad usefulness.

The SDI concept makes possible applications that seek to measure extremely small variations in the extinction coefficient and refractive index of a material. The technique can be used to measure the thickness or refractive index variations of the spinning disk itself. Or it can be used to measure the thickness and refractive index of a material deposited in a spatially periodic pattern on the disk. In the case of an unpatterned disk, the modulation caused by heterogeneity of the disk causes dynamic light scattering.[4] When the disk is used as a substrate that supports a periodic material structure, dynamic light scattering defines the noise floor for the measurement.

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Potential applications of spinning-disk interferometry span the fields of material science (material metrology), microbiology (viruses and bacteria), clinical medicine (immunoassays), homeland security (biological and chemical warfare detection), environmental science (water and air monitoring) and food science (contamination), among others. The central concept of SDI is so basic—conversion of an intrinsically static measurement into a high-frequency optical modulation—that many more applications are likely to emerge. Our current principle interest is the BioCD.

The BioCD

The BioCD is a spinning-disk, self-referencing interferometer that has a layer of antibodies deposited on top of its ridges. Antibodies are recognition molecules that bind strongly to one specific protein (called their antigen) but do not bind to any other molecules. Different antibodies bind to different antigens, and biotechnology can be used to produce antibodies capable of recognizing a broad range of possible analytes. For this reason, clinical assays (such as the PSA test used to detect the prostate-specific antigen) are often based on antibody specificity and are called immunoassays.[5]

The goal of the BioCD is to take the highly static problem of molecular recognition (slow molecule-molecule binding)—ordinarily carried out by detecting fluorescent changes incurred at a fixed spot—and converting the problem to one of high-frequency phase modulation.

When an antibody film of height δh is attached to the ridge in Fig. 1, it modifies the far-field diffraction. The far-field diffraction for the three conditions of land, quadrature and null of Fig. 1 are simulated in Fig. 2(a); the change in

diffraction is shown in Fig. 2(b) when the relative phase between the ridge and the land is changed by $\delta\phi = 4\pi(n\text{-}1)\delta h/\lambda$ where $\delta h = 8$ nm and n = 1.3, appropriate for a monolayer of antibodies. The quadrature condition produces a 4.7 percent change in the diffracted signal.[6] At quadrature, the change is linear in phase, but in the land and null conditions it is quadratic in phase. This makes it possible for small submonolayers to be detected in quadrature, but not in the null interferometer configuration.

BioCD quadrature classes

There is more than one way to perform self-referencing interferometry: all that is required is a reference and a signal wave that can be phase locked. Figure 3 shows two types of self-referencing spinning-disk interferometry defined by their quadrature mechanisms: microdiffraction quadrature (using ridges as discussed above) and adaptive optical quadrature (using two-wave mixing in the far field between the signal and a reference beam inside a photorefractive film).

The trade-off between the two classes of BioCD is the trade-off between the near- and the far-field. The microdiffraction approach requires detailed microstructuring of the disk and careful mechanical tracking by the objective lens, but detection in the far field case is simply by means of an apertured photodetector. The adaptive optical approach has simple disk fabrication and low requirements on head tracking, but requires a sophisticated adaptive optical mixer in the far field. The adaptive approach tends to be more robust, while the microdiffraction approach tends to have higher sensitivity. The technical details of the two quadrature classes are discussed next.

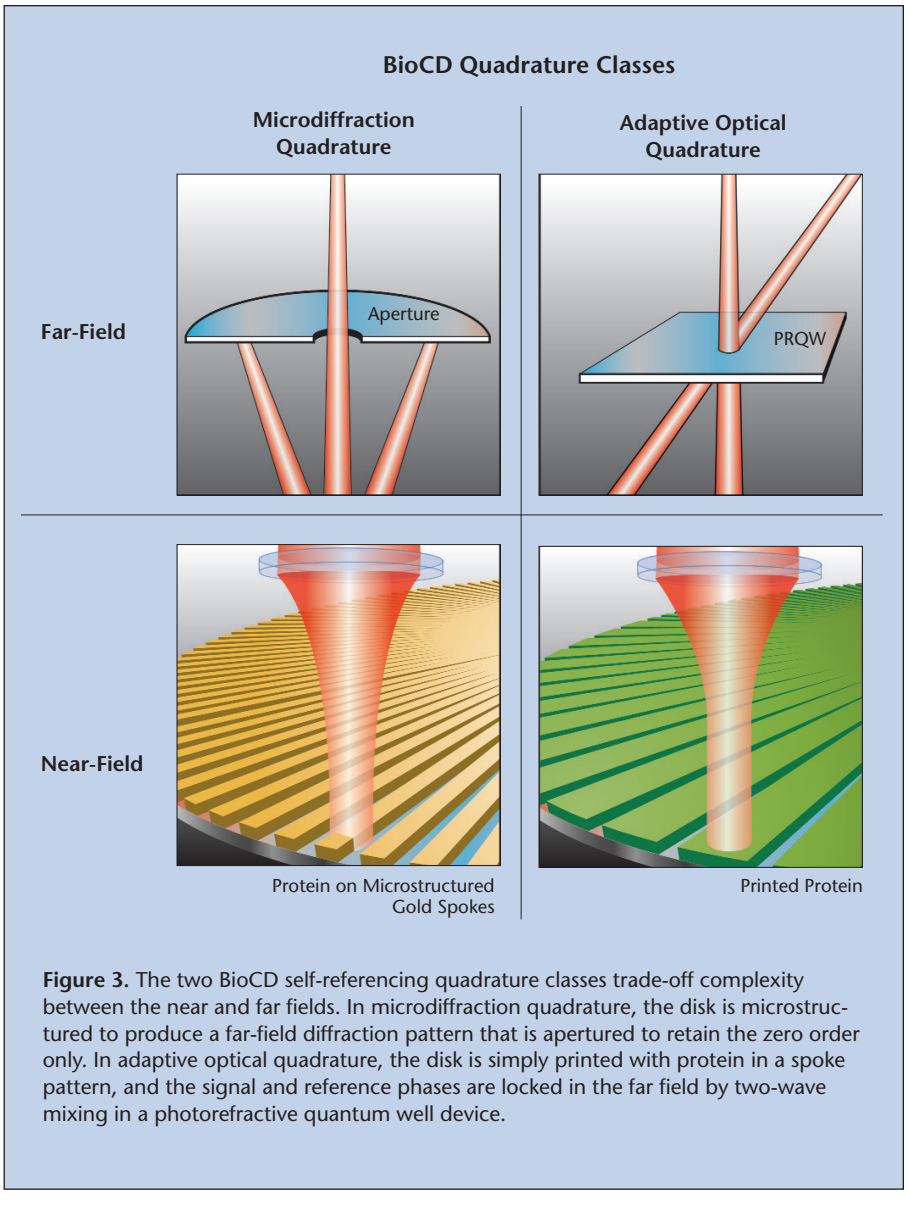

Figure 3. The two BioCD self-referencing quadrature classes trade-off complexity between the near and far fields. In microdiffraction quadrature, the disk is microstructured to produce a far-field diffraction pattern that is apertured to retain the zero order only. In adaptive optical quadrature, the disk is simply printed with protein in a spoke pattern, and the signal and reference phases are locked in the far field by two-wave mixing in a photorefractive quantum well device.

Microdiffraction quadrature

The diffraction of the probe beam from the microstructured ridge plays the role of a wave-front-splitting interferometer similar to Young's double slit experiment. Just as in the case of any two-wave interferometer, the diffracted intensity in the far field along the optic axis has a simple sinusoidal dependence on the ridge height. In this particular case, however, the higher-angle diffraction plays the role of the conjugate port, in which the interferometric response has opposite sign. For this reason the far-field diffraction pattern must be apertured to pass the central diffraction peak, while higher angles are blocked.

To facilitate selective molecule immobilization chemistry on the disk, we deposit a pattern of radial spokes made of gold ridges on a high-reflectance substrate (either a silicon wafer or a laser mirror). In this case, tracks are defined by the selection of the radial distance to the laser spot and require no active tracking. As the disk spins, the laser repetitively samples the land and the quadrature condition, as shown in Fig. 4 for an immunoassay carried out against immunoglobulin.[5] At quadrature, the reflected intensity is approximately 50 percent. When a single antibody layer is immobilized on the gold spokes, the phase of the ridge changes and the intensity at quadrature drops. When the track is exposed to a non-target protein (in this case, a rabbit protein), no binding occurs, and the quadrature signal remains mostly unaltered, indicating a negative test to a non-specific antigen. However, when the track is subsequently exposed to the specific protein (antigen) that is bound by the antibody, the signal at quadrature drops further, indicating a positive test to specific antigen.[7]

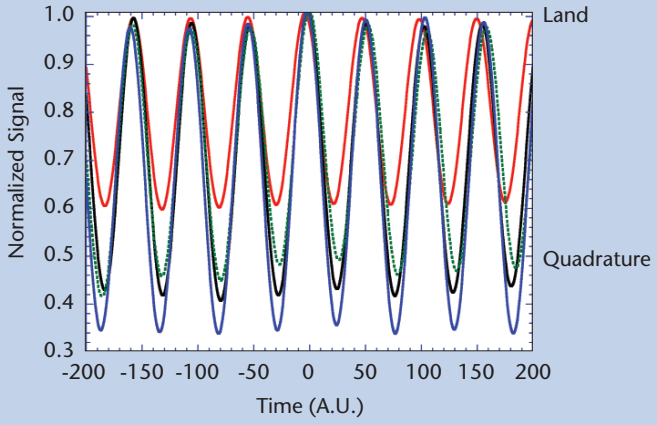

Figure 4. Time trace of a BioCD signal. High reflectance is off the land, while low reflectance is the quadrature condition when the laser spot straddles a ridge (*red trace*). When a layer of antibodies is added selectively to the ridge, the signal drops (*black trace*). Exposing the antibodies to a non-specific protein induces no significant change (*dashed green trace*), but when the antibodies are exposed to and bind a specific antigen, the signal drops further (*blue trace*).[8]

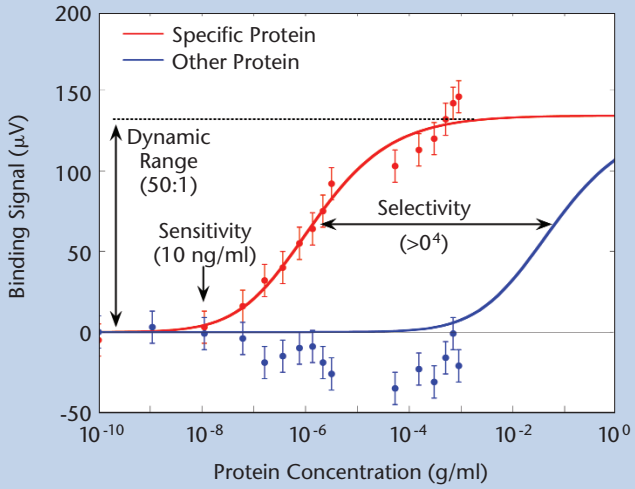

Figure 5. Dose response curve for a microdiffraction quadrature BioCD. The onset of selective binding to a specific immunoglobulin (IgG) protein is at 10 ng/ml and saturates above 1 mg/ml. The non-specific binding has no onset below 1 mg/ml which translate into a chemical specificity of greater than 10,000.

More accurate measurement can be made with the BioCD by changing from time-domain experiments to frequency-domain experiments with much narrower detection bandwidths. This change also allows us to acquire dose response curves for the BioCD. The results are a combination of sensitivity, selectivity and dynamic range, which are indicated in Fig. 5.

The figure shows two dose response curves as raw voltage signal vs. applied analyte concentration from a real-time application of the analyte to the spinning disk.[9] The sample is centrifuged off the disk within approximately 10 seconds, a time period which represents the incubation time for each assay. The signal for specific antigen has an onset at 10 ng/ml (sensitivity) and saturates at 1 mg/ml with a dynamic range of 50:1. The response curve for a non-specific antigen has no discernable onset up to 1 mg/ml, a value which sets a lower bound for the selectivity of the immunoassay at levels greater than 10,000. This number can be roughly translated into the number of equivalent-concentration analytes simultaneously present in a sample that can be analyzed without cross-talk. Alternatively, this number can be translated into the concentration range that can be assayed between two analytes. In this case, a non-specific analyte can be 10,000 times more concentrated than the specific analyte and still not cause false positive readings.

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Adaptive optical quadrature

Locking the relative phase between the reference and signal waves in the far field is an attractive option because it facilitates disk fabrication and tracking. The beams can, for instance, be mixed by use of adaptive interferometry.[10-13] The mixing is accomplished inside a photorefractive quantum well (PRQW) device that performs as a sensitive dynamic holographic film.[14] The phase is locked by the mutual diffraction off the dynamically updating holographic grating; quadrature is selected by the choice of operating wavelength for the laser source.[13] For the adaptive BioCD, protein is printed directly onto glass substrates in a spoke pattern with a spoke width larger than the focal waist of the laser beam. As the disk spins, the printed protein (and the protein that binds to it) impart a repetitious phase modulation on the signal beam.

Slow phase modulation from mechanical vibration and disk wobble is compensated by the dynamic holograms, while the high-frequency phase modulation of the spinning proteins passes through to the detector as intensity modulation.

The protein-printed BioCD disk is incubated (while the disk is not spinning) with different samples around concentric tracks. In our first multi-analyte assay, we printed spokes of protein A and backfilled the surface with protein B. This produced an alternating spoke pattern,

on the same disk, of the two types of proteins. The spokes were exposed to specific antibody A in tracks 2 and 3 and measured, and then exposed to antibody B in tracks 3 and 4 and measured. The final results are displayed in Fig. 6 as two-dimensional (2D) frequency-radius plots.

The data show a strong signal at the carrier frequency of the spokes for the radii incubated with a single specific antibody. Track 1 shows a strong response to antibody A and track 3 shows a strong response to antibody B. Track 2, exposed to both antibodies, shows no signal because both antibody A and antibody B bind to the spokes and cancel out the phase-modulated signal.

These experiments demonstrate the usefulness of adaptive optics applied to an immunoassay, but considerable work is necessary to demonstrate the same l evels of sensitivity and selectivity that have already been demonstrated by the microdiffraction BioCDs. Microdiffraction is simpler, and much closer in concept to conventional CDs. However, the stability of the adaptive approach lends itself more to fundamental quantitative research.

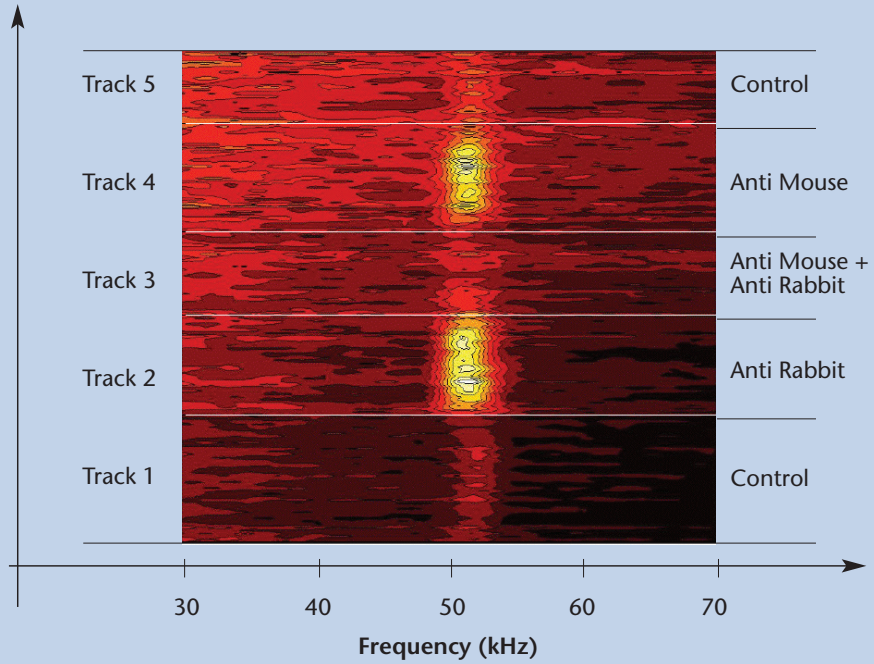

Figure 6. Adaptive BioCD 2-analyte binding experiment. All tracks were dual printed with mouse antigen and rabbit antigen. Tracks 2 and 3 were incubated with anti-rabbit IgG, and Tracks 3 and 4 were incubated with anti-mouse IgG. Selective binding is observed in Tracks 2 and 4, while in Track 3 the two signals cancel.

Future prospects

It is now understood that one's state of health depends on complex protein networks in which concentrations of scores or even hundreds of proteins are interconnected and dependent on each other, often with nonlinear kinetics varying person to person.

For instance, the blood is a kaleidoscopic soup of molecules: hormones, proteins, DNA, sugars, fats, antibodies—over a thousand different types of molecules coming from all parts of the body. Every cell, every tissue, every organ of the body contributes its own characteristic mix of molecules to the blood. Each mixture is like a signature or a fingerprint. To gain a more complete understanding of where the state of health of an individual is today and where it will be next year, or how it will respond to a drug, requires an analytical assay like the BioCD that can test for hundreds or thousands of analytes.

The challenge for such a global assay technology is to measure hundreds or thousands of trace concentrations with high specificity and large dynamic range—which we have already begun to demonstrate. For point-of-care testing it is also necessary for the technology to be fast, cheap and reliable. If the BioCD can satisfy these requirements, it could replace the current paradigm of clinical testing in outside labs and bring testing to the point of care in doctor's offices and in the home, to become a powerful tool for diagnostic and preventive medicine.

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