Silica microspheres enable sensitive DNA detection

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A DNA hybridization detection method based on changes in optical resonance of oligonucleotide-coated silica microspheres may eventually be incorporated into a highly sensitive, fluorescence-free DNA microarray system.

Now that scientists have sequenced the genome of many species, the effort has begun in earnest to scrutinize and use the wealth of information. Most of these analyses involve sifting through DNA to find specific sequences, and promise to answer questions in the fields of medical diagnostics, drug discovery and forensics, to name a few.

In the past 10 years, scientists have developed a plethora of DNA screening tools, most of which are photonics-based. Now, a team of researchers from Rockefeller University and Polytechnic University, Brooklyn Campus, both in New York, has added a new method to the mix: a highly sensitive, label-free way to detect and quantify DNA based on changes in the optical resonance of micron-scale silica sphere probes.

Most DNA screening tools use oligonucleotide probes — short sections of known DNA sequences — that hybridize to complementary strands in a sample. In most commercial DNA microarrays, the probe, the sample — or both — are labeled with fluorescent tags and examined using an image acquisition and analysis system. These devices enable high-throughput DNA screening because of their multiplexing capabilities. However, detecting how much of a specific DNA sequence is present in a sample is difficult. Furthermore, the use of fluorescent tags is not always desirable or feasible, depending on the application.

Another method of hybridization detection is based on a phenomenon called surface plasmon resonance. In this case, an optical substrate is covered with a thin metal film to which oligonucleotides are attached. Simply put, when the surface probe DNA hybridizes, it changes the reflectivity characteristics of the metal film, which can be detected optically. This method does offer highly sensitive and real-time DNA detection and quantification, but does not yet boast the same multiplexing capabilities as fluorescence techniques.

The New York researchers purport that their method has the multiplexing potential of fluorescence techniques but, like surface plasmon resonance, has increased sensitivity and does not need fluorescent tags. It is based on exciting optical resonances in micron-scale silica spheres, whose surfaces are coated with an oligonucleotide-hydrogel matrix.

The spheres have about 200-μm radii and are dielectric. They can confine light for long periods via total internal reflection, producing a resonance at certain wavelengths known as a whispering gallery mode.

Figure 1. The DNA detection scheme devised by researchers from two New York universities uses standard telecommunications components. The system detects DNA hybridization based on changes in optical resonance of a microsphere coupled to an optical fiber transmitting IR light.
Some members of the research team had previously shown that the addition of other dielectric molecules, such as proteins, to the surface of a sphere causes an optically detectable shift in its optical resonance frequency toward higher wavelengths. They have shown quantitatively that this occurs because the surface molecule polarizes when in contact with the sphere’s evanescent field. Thus, they suspected that a DNA hybridization event on the sphere’s surface would likely produce the same effect because DNA also has dielectric properties.

In the September issue of *Biophysical Journal*, the researchers described their process for designing the probes and testing them as part of a hybridization detection scheme. They melt the tips of 125-μm-diameter single-mode near-IR optical fiber from Corning Inc. of Corning, N.Y., into spheres. Then they coat the spheres with oligonucleotide probes and couple them evanescently to a single-mode optical fiber. This procedure involves chemically eroding the optical fiber into its core along a small portion of its length so that the spheres can access the evanescent field of the light as it travels down the fiber (Figure 1).

In most cases, they coupled two spheres containing different oligonucleotides to a single optical fiber. This allowed them to use one as a control in each experiment to ensure that only a specific DNA hybridization event caused a resonance shift in the spheres. The researchers held the spheres in place relative to the etched fibers using separate X-Y-Z stages.

A tunable laser diode manufactured by the Optoelectronics Div. of Mitsubishi Chemical Corp. in Tokyo is coupled to one end of the optical fiber, while an InGaAs photodetector from Thorlabs of Newton, N.J., is coupled to the other. The laser emits infrared light into the fiber, through the spheres and to the detector, which records resonances as a “dip” in the spectrum of the transmitted light intensity. A data acquisition card and LabView software from National Instruments Corp. of Austin, Texas, digitally records and analyzes the intensities. The software identifies changes in the intensity spectrum and displays them as separate dips for each sphere.

When the surfaces of the spheres are identical, their resonances shift by the same amount. However, when a hybridization event occurs on one of the spheres, a differential shift of a few hundredths of a nanometer occurs. The signals from two spheres can be distinguished because the narrow sphere-specific resonances are separated by a large enough number of wavelengths to pick out the individual perturbations.

To test the detection system, the researchers placed two spheres in a sample cell, into which they subsequently injected DNA strands complementary to the oligonucleotide on just one of the spheres. They detected an increase in the resonance wavelength of the complementary sphere, but not in the one with the noncomplementary DNA probe.

They also tested their technique using another standard: single nucleotide mismatch detection. Vollmer said that most methods are not sensitive enough to indicate if one or two of the base pairs do not match perfectly in a hybridization event. They created two spheres with DNA probes differing by a single base pair, and...
added DNA that was perfectly complementary to only one of the probes. They detected almost a tenfold increase in resonance wavelength in the sphere with the perfect match compared with the sphere with a single nucleotide mismatch, with a high signal-to-noise ratio.

They have not yet tested the lower limits of sensitivity in terms of DNA concentration in solution; however, using the measured resonance shift, they calculated that the experimental limit of their detection technique is about 6 pg of DNA per square millimeter of sphere surface. According to Vollmer, this trumps the highest sensitivity demonstrated in other methods — about 10 pg/mm².

He said that the high detection sensitivity stems directly from the spheres' high Q-factor, which describes the resonant quality of a material. "Light interacts with a surface molecule many times because it stays much longer inside the sphere. The higher the Q, the higher the sensitivity." He also said that there is a trade-off in that smaller spheres produce a larger and more easily detectable resonance shift, but that they also cause a reduction in Q-factor and, therefore, insensitivity.

The researchers believe that by better engineering their apparatus, they might eventually achieve single-molecule detection sensitivity. "We use standard telecommunications components right now," Vollmer said. "We chose the IR wavelength because that's what all the components were designed for. It's not actually the ideal wavelength, though. We hope to change it to about 550 nm, where Q is higher due to lower absorption by water."

In addition, the researchers are trying to simplify the evanescent coupling step by using flat instead of fiber waveguides, which may ultimately allow them to integrate the device on a chip. They also are exploring ways to further multiplex their scheme and attempting to detect other biologically important molecules, such as proteins, bacteria and viruses.