Multiplexing the Raman Way
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On a long journey by sea from London to Bombay, the physicist C.V. Raman spent his time gazing at the ocean blue. Unsatisfied by a common explanation that the blue color was a reflection of the sky, he went on to show that the scattering of sunlight by water molecules gave the sea its color. His discovery of this phenomenon, named the “Raman effect,” supported the theory of the dual nature of matter and won him the Nobel Prize in 1930—the first such prize awarded to an Asian in the sciences. Today his science underlies a powerful diagnostic modality known as “surface-enhanced Raman spectroscopy” (SERS),1 which could make multiplexed assays easier and more affordable. Clinical Chemistry spoke with 2 scientists who have been playing a leading role in establishing this technology: Dr. William C. Wilson, research microbiologist at the Agricultural Research Service Center for Grain and Animal Health Research in Manhattan, Kansas, and Dr. Patrick A. Johnson, assistant professor in the Department of Chemical and Petroleum Engineering at the University of Wyoming. Dr. Dan Milner, Assistant Medical Director of Microbiology, Brigham and Women’s Hospital, Boston, gave views on the clinical utility of this technology.

Why Is This Invention Important?

Although most diagnostic platforms are designed to perform tests one at a time, this technology is well suited for multiplexing. Raman spectroscopy shows unique signatures for the dyes that are used, and the approach uses metallomagnetic beads that can conjugate with a number of substrates, from oligonucleotides to antibodies. “I am seeing a trend moving towards syndromic surveillance,” says Dr. Wilson, elaborating that this technology is suited for running 10–20 tests at once. A key feature of SERS is that it shows a massive enhancement in Raman intensity, on the order of $10^{10}$, when the target is near a metal nanoparticle. This feature saves on the need for the enzymes required for amplification in PCR assays.

Of further importance is the high sensitivity of the technology, with a limit of detection in the nanomolar range, as Dr. Wilson and Dr. Johnson demonstrated in a SERS immunoassay they designed to detect West Nile virus and Rift Valley fever virus (1). Raman dyes are used in SERS, and they have a number of advantages over fluorescent sensing tags that make them well suited for multiplexing. They are less susceptible to photobleaching, and multiple, non-overlapping Raman dyes are available. These dyes also have a low cost, supporting the affordability of the assays.

How Does It Work?

A typical SERS immunoassay design includes 2 main components. The first component is a gold nanoparticle (GNP) coated with a Raman dye or reporter. The second component is a paramagnetic nanoparticle (MNP) that allows separation of the complex in solution by use of a magnet. For each antigen being detected, the GNP has a unique Raman dye and the GNP and MNP are conjugated with a polyclonal antibody specific to the antigen (Fig. 1). A Raman spectrometer with a 785-nm laser is used to

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3 Nonstandard abbreviations: SERS, surface-enhanced Raman spectroscopy; GNP, gold nanoparticle; MNP, paramagnetic nanoparticle.
excite the complex, and the Raman spectrum is simultaneously acquired and analyzed.

C.V. Raman could not have predicted that decades later we would be applying his work to diagnostic assays. The underlying principle of the Raman effect can be explained through quantum mechanics. When a laser hits a sample, the energy of the incident photons may not be sufficiently high to excite the molecules to a stable electronic state. In that case, the excited electrons visit a virtual state and then fall back to the ground state. Most often, very little energy is transferred to the electrons; hence, the incident light and the emitted light are of the same energy and color. This phenomenon is called elastic scattering. In the less common but more important inelastic scattering, the incident light and the emitted light are of different colors, because the electrons expend kinetic energy through vibration. The Raman effect is of this type, and the behavior of the electrons is stereotypical to the nature of the sample. Thus, an analysis of the change or shift in spectrum gives a fingerprint of the sample being analyzed.

When asked what makes their methodology unique given the long history of research in this area, Dr. Johnson emphasized 2 points. The first is that the recognition elements in their assay bind in solution rather than on a surface. This feature helps reduce assay time and increase sensitivity. The second is the simplicity of their method. The analyte is sandwiched by the antibodies of the GNP and MNP, and the complex is separated by a magnet. SERS then recognizes the bound Raman dye.

**Where Can This Technology Fit in the Clinical Laboratory?**

Dr. Milner sees the most important application for this technology in the detection of events occurring at very low concentrations. “Low copy number (of antigen or nucleic acid) situations would be ideal, especially when the volume of sample is limited. Particularly important designs could include, but are not limited to, screening blood products for
infectious agents and detection of pathogens in blood, plasma, CSF [cerebrospinal fluid], or urine.”

Dr. Milner recognizes the need to address a limitation of the current technology, the high complexity of interpreting test results. “For tests to move from the bench to the bedside, the interpretation will need to be a bit more automated and any inconclusiveness sorted out with an appropriate follow-up algorithm.” He goes on to give an example. “With any antibody detection assay (e.g., HIV antibody), there is an ‘indeterminant’ area of the technology between an absolute negative and positive. If SERS can eliminate this area and provide a clear yes/no cutoff, it would be superior to all existing antibody detection assays and thus more quickly adopted.” By harnessing the power of multiplexing, Dr. Milner believes that if SERS can capture the diversity of the polymorphisms of a gene by detecting mRNA, the technology could prove superior to sequencing in cost-effectiveness.

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