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INSIGHTFUL LIGHT

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Raman spectroscopy may offer doctors, dentists and forensic scientists a better tool for molecular detection

From CT, PET and MRI to the original X, a vast alphabetical arsenal of tools tells doctors what is going on inside the body. But despite their successes, these tools often fail to detect the subtle changes that signal the imminent onset of illness. Mischief at the molecular level often evades doctors' current imaging and detection abilities. So for sensing such changes, biomedical scientists are taking a tip from chemists. Using a method known as Raman spectroscopy, medical detectives are moving ever closer to exploiting the power of light to improve disease detection.

Long used in labs, spectroscopy employs light and other types of electromagnetic radiation to analyze matter. The various spectroscopic techniques reveal a molecule's unique chemical fingerprint by measuring the wavelengths of light that the molecule absorbs or emits, or by tracking how radiation scatters after interacting with a molecule. For 30 years, scientists have been eager to harness the power of Raman spectroscopy, a type of scattering spectroscopy, to image the body at the level of individual molecules. The method holds promise for pinpointing the beginnings of dental cavities and tumors. And it could even help forensic investigators nab killers sooner by lifting latent fingerprints from corpses.

A variety of researchers, from dentists and doctors to chemists, now report some of the first successes using Raman spectroscopy to probe chemicals and minerals within and on living — and dead — bodies. "Raman spectroscopy is a very powerful tool," says Cristina Zavaleta, a molecular imaging radiologist at Stanford University. But, she adds, the technique still needs some time to develop.

In recent years, scientists have rapidly overcome many of the hitches holding up the widespread use of Raman-based instruments. That progress leads many to speculate that within a few years doctors and dentists could be wheeling new, Raman-based tools into the examining room, or detectives could even be driving them to the scene of a murder.

Imaging humans' insides

In Raman spectroscopy, scientists shoot a laser light at a target molecule and measure how the wavelengths of scattered light, in the form of photons, coming off the target compare with the laser's original wavelength. Only one in 10 million of the photons hitting the target shows an increase or decrease in wavelength. Detecting these rare photons is the challenge — and ultimately the payoff — for scientists seeking to harness the Raman effect for clinical applications.

The wavelength change is called the Raman effect in honor of Indian physicist Chandrasekhara Venkata Raman, who first showed in the 1920s that measuring the changes in wavelengths of scattered photons can help scientists identify a compound's molecular makeup. He won the Nobel Prize in physics in 1930 for his work. Currently, geologists, chemists and archaeologists use the technique to study minerals in the soil, identify new materials and determine the pigments in ancient paintings, manuscripts and other artifacts.

"At this point, Raman spectroscopy is good for surface scans," says David Batchelder, a Raman researcher from the University of Leeds in England. Unlike X-rays and CT scans, existing Raman tools have yet to let doctors look inside the body. "To penetrate deep into tissues," Batchelder says, "the equipment has to be very good."

But Stanford University researchers, including Zavaleta, are on track to engineer inward-probing Raman tools. The key, the scientists discovered, is in using nanoparticles. By wrapping cancer antibodies around gold nanoparticles, the team used Raman spectroscopy to detect tumors in a living mouse.

Zavaleta and colleagues injected the nanoparticles into the mouse. Each specific antibody attached to a specific type of tumor cell. When the researchers shone laser light across the animal's body, the cells with attached antibody-coated nanoparticles showed a change in wavelength compared with the laser.

Signals coming from the antibodies are very weak, Zavaleta says. But the gold in the nanoparticles boosts the signal because the laser excites the gold cores and the metal actually shows an intensity increase in its surrounding electric fields. The Stanford team scanned the mouse's body for the excited electric fields and pinpointed the locations of the nanoparticles using a Raman microscope.

The microscope looks like a standard optical microscope. But researchers added the laser and a sensitive detector to the instrument to read the spectral fingerprints of the nanoparticles and then compute where in the body there were excited electric fields and changes in photons' wavelengths. Ultimately, the team's device formed an image of the mouse's internal tumors.

And, because the injected nanoparticles attached to different tumor types, the scientists were able, in one scan, to identify where different cancer cells were in the mouse's body. That single scan for many types of cancer is the novel aspect of this research, Zavaleta says. She and her colleagues reported their progress in the April 15 *Proceedings of the National Academy of Sciences*.

Aside from CT scans and X-rays, doctors are using fluorescence imaging with quantum dots to take a peek at the finer details of the human body. But the Raman technique, Zavaleta says, could exceed the capabilities of quantum dots. Doctors would need to inject only one one-thousandth the number of nanoparticles required for imaging using quantum dots. Showing that scientists can image living subjects with fewer nanoparticles has never been done successfully before, the Stanford radiologist says.

Oncologists could eventually use Raman spectroscopy during surgery to scan diseased tissue. Injecting the new nanoparticles or a variation of them into the body during an operation would show surgeons where the tiniest abnormal cells are just beginning to form. The surgeon could remove these developing cancer cells and perhaps prevent future growth and spread of the tumor, Zavaleta says.

Raman spectroscopy could also replace visual checks for tumors and diseases like cervical cancer. "In Pap smears doctors just look for cancer cells," says Batchelder, "but certain types of tumors are hard to identify. Raman technology could pick out the particular molecular processes related with this type or a particular type of tumor, making it easier to catch."

The developing technique, though, will never completely replace PET scans, MRI, ultrasound and other imaging methods, Zavaleta says. Each technique brings its own advantages to figuring out what's going on inside the body. Yet some doctors are trying to rid their offices of X-ray machines, at least the doctors that poke at people's teeth.

No drilling for the dentist

In addition to ridding the body of cancer cells, Raman spectroscopy may rid dentists of their drills.

No one likes having cavities filled. So, to avoid putting patients "under the drill," Lin-P'ing Choo-Smith and her colleagues at the National Research Council Canada's Institute for Biodiagnostics in Winnipeg are studying how to use Raman spectroscopy to spot cavities much sooner than currently possible.

Working with extracted teeth, the Canadian dental researchers have detected tiny cavities by using Raman spectroscopy to search for slight decreases in calcium hydroxyapatite, the dominant mineral component of teeth. The team presented its latest work at a meeting in June sponsored by the European Organisation for Caries Research and then discussed it again in July at a conference of the International Association for Dental Research.

Cavities, which often result from dental caries, are spots on the tooth where minerals have leached out. Bacteria in plaque play a key role in cavity formation by producing acids that leach the minerals. With less minerals in the tooth structure, the tooth begins to dissolve and can rot.

Dentists usually use X-rays and dental probes — the metal picks that can scratch at the teeth — to detect cavities. But with these tools, dentists can detect only major damage to the tooth and cavities as big as a millimeter in size. And by this stage, Choo-Smith says, the tooth can be in pretty bad shape.

"Using Raman, however, would let dentists detect small changes in mineral levels of the tooth long before a cavity actually became a cavity," Batchelder says. Dentists could detect precursors to cavities and weak spots with lesions only 100–250 micrometers deep, about the size of an individual grain of sand. The Raman tool might also detect troublesome spots between teeth.

Catching the problem areas at an early stage could eliminate the dentist's need to drill, Choo-Smith says. Instead patients could self-treat the tiny, trouble areas with fluoride or antimicrobials.

Using spectroscopy coupled with an imaging technique called optical coherence tomography to detect a speck of a cavity might seem like overkill to some patients, says Cecilia Dong, a dentist at the University of Manitoba in Canada and one of Choo-Smith's collaborators. But the more information dentists have, the more accurate their diagnoses will be. That could mean less pain for patients, she adds.

What's more, Raman spectroscopy does not use ionizing radiation like X-rays do, so pregnant women and small children could be safely scanned, Dong notes. With no

radiation exposure to worry about, dentists could use Raman testing every time a patient comes into the office. Frequent scanning, she says, will truly show dentists and hygienists who is doing their daily brushing and flossing.

But adapting Raman spectroscopy for the dentist's office, Choo-Smith explains, would require that dentists have a portable Raman-based unit and a miniature wand or probe to use in the mouth. Engineering and manufacturing probes for reading scattering spectroscopy emissions, specifically ones small enough to scan a tooth, is one of the greatest challenges for current Raman spectroscopy researchers.

And, while ever-smaller fiber optic cables and, in medicine, nanoparticles may help scientists add Raman spectroscopy to their disease-detecting arsenal, the development work is far from over. Still, each round of probe design and research yields clearer results. Within the next year and a half, Choo-Smith expects to take prototype probes into dentists' clinics for testing. "I think it will still be another three to five years before they will have a product to wheel into their examining rooms," she says.

Lifting latent prints

From inside the body to inside the mouth, Raman spectroscopy shows promise for detecting the molecular fingerprints of disease. But it also could prove useful for identifying real fingerprints — such as the prints a killer leaves on a victim's body.

"Prints are really hard to lift from corpses," says Linda Lewis, a chemist at the Oak Ridge National Laboratory in Tennessee. "Our goal, though, is to detect fingerprints on surfaces where they are not traditionally detected."

Lewis is developing a device based on Raman spectroscopy that would enable detectives to trace the chemical signatures of certain residues left by human hands — on corpses or even hard-to-analyze evidence. Working with researchers at ChemImage in Pittsburgh, Pa., and the U.S. Naval Research Laboratory in Washington, D.C., Lewis is using silver-coated nanowires, similar to Zavaleta's gold nanoparticles, to mark a killer's prints, or at least, right now, human prints left on dead animal skin.

The nanowires target specific fingerprint components — such as eccrine, a watery substance that comes out of the pads of human fingers and is not well detected using current forensic methods — that give off Raman scattering emissions. "The most active signal we get right now is actually from urea," she says.

In theory, Lewis says, detectives would spray the silver-coated nanowires onto a corpse in the field and then use a Raman microscope-laser device to scan the body. The nanowires would detect particular molecules in urea, eccrine and possibly other substances. Passing the laser over the body would trigger the silver coating on the nanowires to amplify the signals emanating from the laser's scattered photons by changing the electric field. Using the microscope, which would register the chemical spectrum and locate the signals, the investigators could isolate a killer's fingerprint.

Lewis says analyzing the Raman spectroscopy scans is similar to looking at the individual pixels from a picture. Not every pixel has high peaks of light on it. Similarly, not everything gives off a Raman scattering signal. When the pixels are put together, though, the image appears in a matrix of light and dark spots. On the skin, the scattering signals from the 1-by-1-inch laser-light blocks can come together to show a fingerprint, like the pixels show the image, she says. And, once the Raman tool reveals the location of the left-behind molecules, detectives could collect the print for further analysis, just as they do now from hard surfaces.

Lewis and her colleagues are currently writing up their early results on the spectroscopy device for submission to the *Journal of Forensic Sciences*. Her team next needs to look for prints on actual decaying bodies instead of preserved pig and human skin, she says. Scanning for prints will help her team design a Raman spectroscopy device that could detect killer's prints left on bodies found 24 to 48 hours after death.

"We need to see if the prints decompose as the body does or if heat or other factors affect the signals we can get from the prints," she says.

And, although the Raman spectroscopy print identification tool is still in its testing phase, Lewis says the team wants to have something ready to go in about two years.

But corpses are not the only crime scene evidence detectives could scan for the signatures of fingerprints. Investigators could also do Raman-based analyses on explosive residues from terrorist attacks or even on heavily contaminated drug evidence. "Prints are hard to lift from these places too, and we want the device to work on all tough surfaces," Lewis says. "My far out vision, probably in 10 years, though, is to scan live skin. That would identify abuse criminals."

But creating a forensics Raman tool for widespread use means engineering nanowires more efficiently and at a lower cost. Researchers at the Naval Research Laboratory can make small quantities of nanowires, with a lot of effort, Lewis says. "The challenge is making large batches of the silver nanowires" more quickly, she says.

Medical applications for Raman spectroscopy, Batchelder notes, face similar delays. Don't

expect to see Raman tools in a dentist's or doctor's office tomorrow, he says, adding that while he has seen technology improve immensely in the past 10 years, each biomedical application for Raman-based tools has had its holdups.

For medical researchers, probe design is a struggle. No commercial companies are currently invested in developing the probes, even though there is a major market for them, dental researcher Choo-Smith says. Researchers are basically going it alone, trying to build something that will bring the sensitivity of Raman spectroscopy to the examining and operating rooms. Still, scientists and doctors are optimistic, and while they recognize the obstacles, most are confident that soon they will be able to add an "R" for Raman spectroscopy to the alphabetical arsenal they use to explore the human body and catch criminals.

Editor's note: David Batchelder, formerly of the University of Leeds, died on June 6.