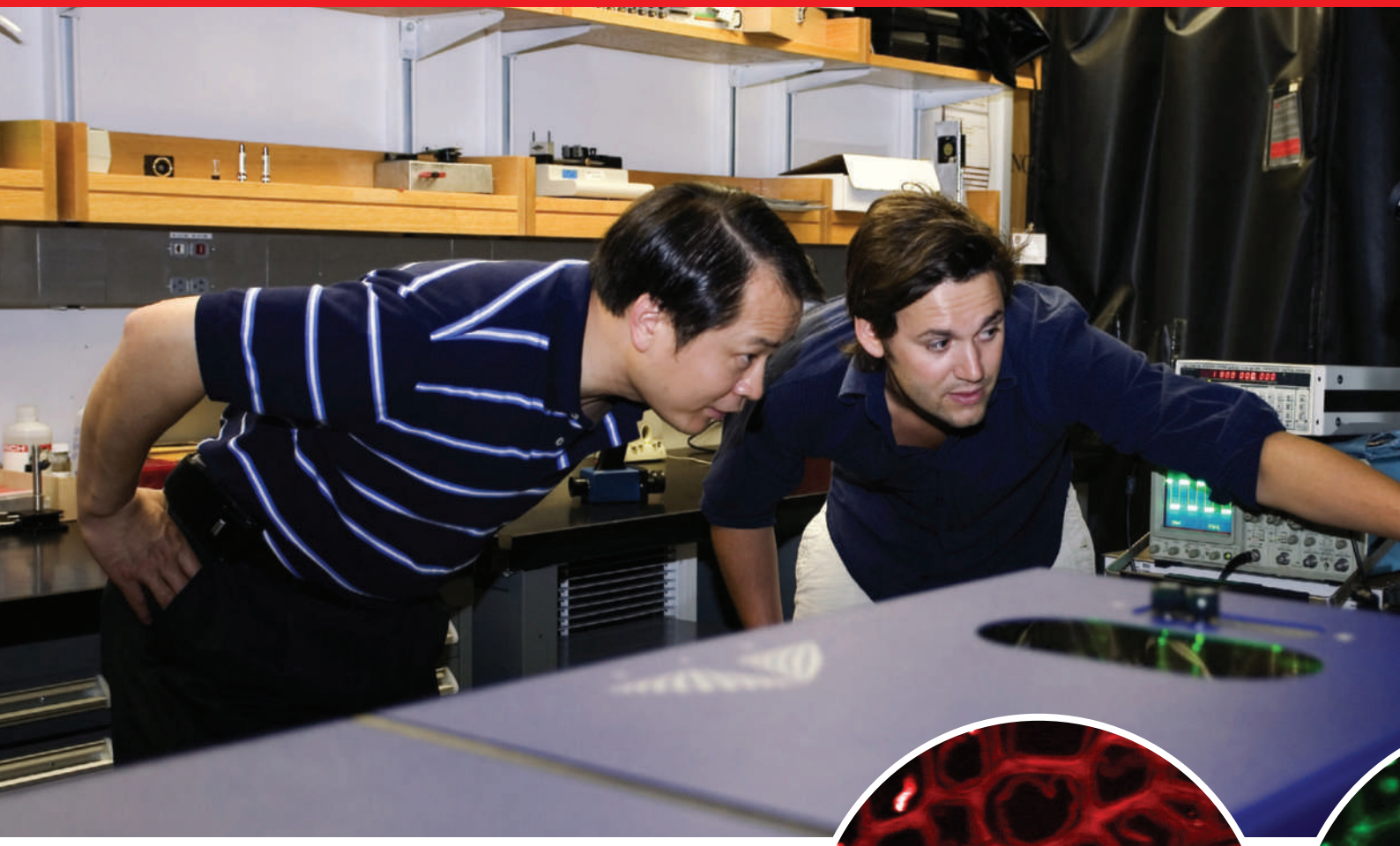
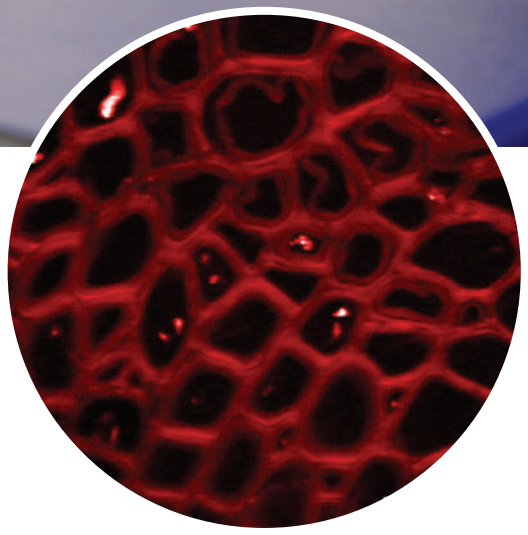
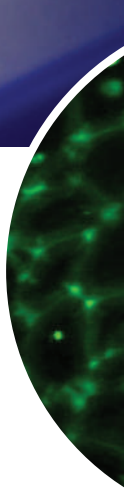






U. LOSTER

THE NAKED MICROSCOPE

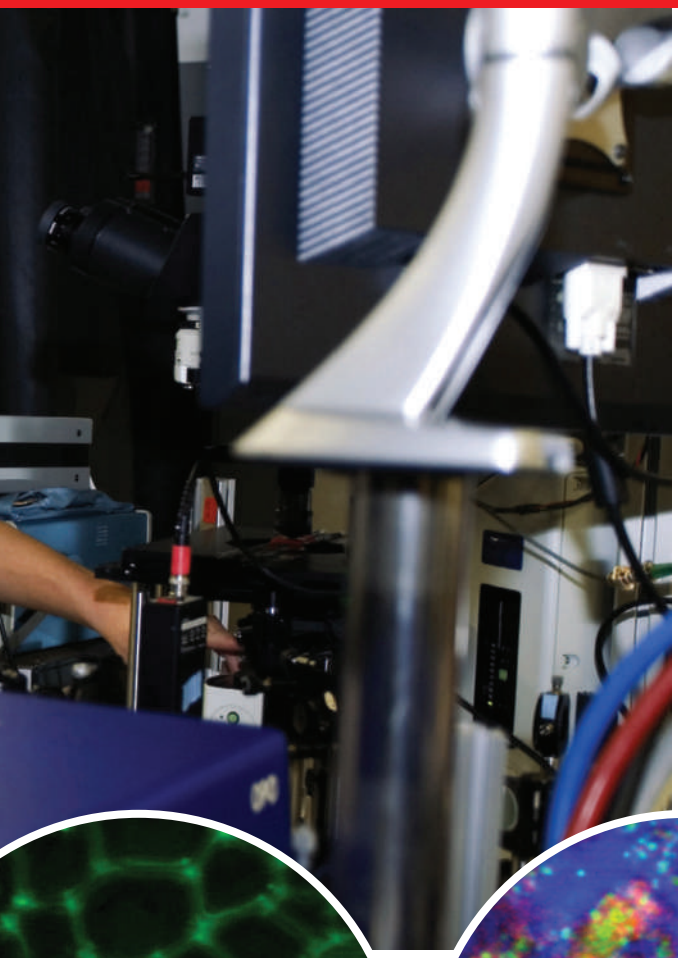
Sunney Xie's newest microscopes don't look like the latest in sophistication. Tucked away in his biochemistry lab at Harvard University, they seem to be ad hoc assemblies of lasers, objectives and electronics, surrounded by a thicket of optical equipment. "Don't worry about most of these," says graduate student Christian Freudiger, gesturing to the latest addition to Xie's microscope family. "You only need a few optics to use the microscope. The rest are just for us to play with." As he leans briefly on the table, which is designed to eliminate vibrations, it counterbalances his weight with a reproachful "shhhh".

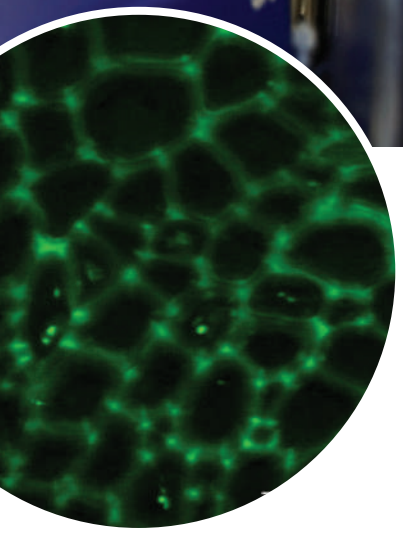
Freudiger and the other researchers 'playing' in Xie's lab have pioneered techniques to see biological molecules in their natural state. Based on Raman spectroscopy, the methods detect compounds from the characteristic vibrations of their chemical bonds, and they free the user from having to label molecules by attaching tags such as gold particles, antibodies or fluorescent proteins. Many researchers are unwilling to abandon labels because they offer an unparalleled ability to distinguish target molecules in a cell. But for others, who want to observe a molecule 'naked', without the interference of a molecular tag, it is proving to be hugely liberating.

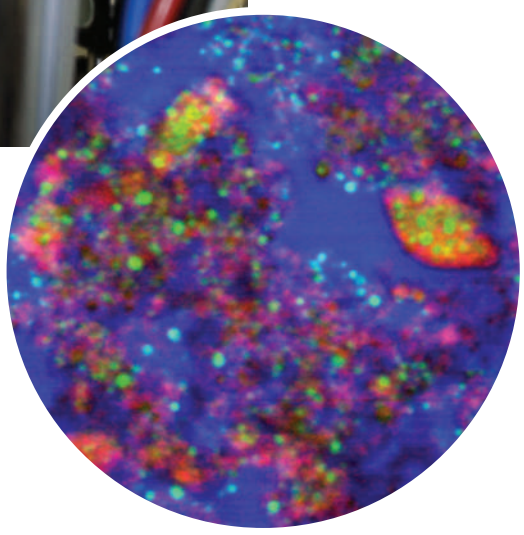
In traditional Raman microscopy, laser beams illuminate a sample and the characteristic shift in wavelength caused by chemical bonds helps researchers pinpoint the identity and location of certain molecules. The method is therefore best suited for imaging molecules with distinct spectroscopic properties, such as lipids. But in early Raman microscopes the signal was weak and the technique required long exposure times (sometimes more than a day), precluding the possibility of monitoring biological processes that happen in minutes, seconds or less. The microscopes also relied on powerful lasers, which fry delicate biological samples.

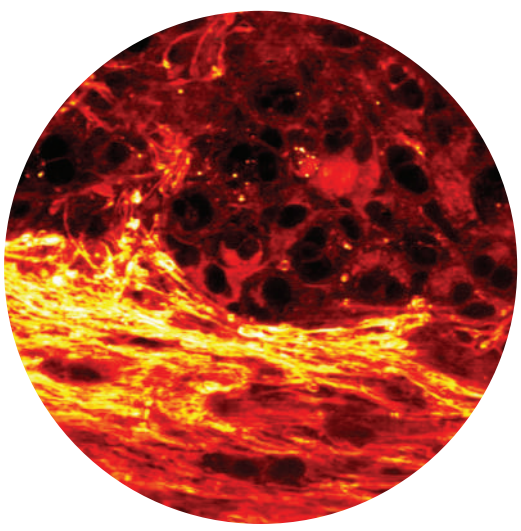
About a decade ago, Xie's lab developed a method called coherent anti-Stokes Raman scattering (CARS) microscopy, which uses two laser beams to excite molecular vibrations and generates a stronger signal. This technique cut down on exposure time and laser power, but was plagued by a high background signal.

Then in December last year, Freudiger together with postdoc Wei Min reported a further improvement[1]. Called stimulated Raman scattering microscopy (SRS), the method excites molecules with two laser beams that have been cali-









B. G. SAAR, Y. ZENG; C. FREUDIGER, G. VAN DALEN, M. VAN RUIJVEN, C. XIAO; C. FREUDIGER, G. YOUNG, S. KESARI

Sunney Xie (left), Christian Freudiger and their label-free microscope, which has resolved (from bottom left) cellulose; lignin; water (blue), protein (red) and oil (green) in a soya drink; and lipids in brain tumours.

brated so that the difference between the frequencies of the beams matches the vibrational frequency of the molecule to be imaged. The result: only the target molecules are excited and the troublesome background is squelched. By eliminating the high background signal, the SRS technique promises to extend label-free microscopy to molecules that couldn't previously be detected.

Biologists are already lining up to give Xie's microscopes a try, along with similar instruments being developed by other groups[2,3]. Biofuels researcher Shi-You Ding of the National Renewable Energy Laboratory in Golden, Colorado, wants to use SRS in his pursuit of techniques to degrade cellulose, a major component of plant cell walls, into smaller sugars that can be used for fuel. His research has been frustrated by the lack of a method to distinguish cellulose from lignin, another molecule found in plant cell walls, without having to stain cells with techniques that affect the distribution of the two compounds. "Even if you want to tear down a building, you need to know what the structure is," says Ding, "but in this case there was just no way to see it."

"Biologists have never been able to get this kind of information before."
— Shi-You Ding

Ding teamed up with Xie's graduate student Brian Saar to create movies of cellulose and lignin dynamics, allowing him to find chemical conditions that will break down lignin and leave the cellulose intact. "It's a very powerful tool," Ding says. "Biologists have never been able to get that kind of information before." Xie's group is also collaborating with researchers at the pharmaceutical company Pfizer in New York, who have already used the technique to track the acne-treatment retinoic acid as it is absorbed by skin. Scientists at Unilever based in the Netherlands and the United Kingdom want to use SRS to study the effects of cosmetics on skin and the distribution of fats and proteins in foods without having to attach bulky labels.

At the moment, an SRS microscope costs in the order of half a million dollars, but Xie hopes that technological improvements will reduce the cost and complexity of the set-up. Zeiss and Leica, two major microscope manufacturers, have just licensed the technology, and aficionados who have set up their own CARS systems can tweak their microscopes to accommodate SRS, he says.

SRS still has a few kinks to iron out, says Jing Kang, a professor of medicine at Harvard Medical School. It should enable researchers to distinguish between different types of unsaturated fatty acids at biological concentrations within the cell — discerning heart-friendly omega-3 fatty acids from the less-healthy omega-6 fatty acids, for example. But so far, Kang and his collaborators in the Xie lab have not been able to do so. "In theory it should be okay," Kang says, "but whether it's really going to make it — that's another story." ■

Heidi Ledford

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2. Ozeki, Y., Dake, F., Kajiyama, S., Fukui, K. & Itoh, K. *Opt. Express* **17,** 3651–3658 (2009).
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See also page 629 and online at http://tinyurl.com/microspecial.