SINGLE MOLECULE

The dyes that came in from the cold

Three groups achieve room-temperature, single-molecule detection of nonfluorescent, photon-absorbing compounds.

Scientists have made steady progress in molecular-scale detection since the mid-1970s, with Tomas Hirschfeld's pioneering efforts to observe individual fluorescently tagged proteins. Fluorescence imaging, however, is blind to many compounds. "Not all molecules are fluorescent," says Leiden University's Michel Orrit, "and fluorescent molecules often blink and go into nonfluorescent states."

One alternative is to analyze samples based on photon absorption, and in 1989 William Moerner achieved a breakthrough by using lasers tuned to pentacene's 'zero-phonon line', a narrow wavelength range at which absorption increases dramatically. He performed these experiments at temperatures near absolute zero, and 'warming up' absorption analysis has been seen as a near-insurmountable challenge, as photon absorption drops dramatically and weak signals get overwhelmed by scattering noise arising from sample heterogeneities. However, three new papers from Orrit, Harvard University's Sunney Xie and Vahid Sandoghdar's group at the Swiss Federal Institute of Technology (ETH) Zurich demonstrate a variety of approaches that offer a foundation for room-temperature, absorption-based microscopy.

Orrit's team exploited a method known as photothermal contrast, in which gold nanoparticles and organic dyes are targeted by a pair of lasers (Gaiduk *et al.*, 2010). The first laser is tuned to a wavelength that is absorbed by and heats the target molecule; this heat dissipates from the target into its immediate surroundings, changing the index of refraction for the second, 'probe' laser, tuned to a wavelength to which the target is transparent. This yields a subtle but detectable signal without blinking or notable bleaching and with minimal photon noise.

Xie adopted an approach that builds on his group's experience in working with nonlinear optical phenomena such as stimulated emission. Their setup uses a modulated 'pump' laser tuned to a wavelength that excites the target, reducing the quantity of ground-state electrons that can be excited by the continuous 'probe' laser (Chong *et al.*, 2010). The two beams are close in wavelength, but filtering enables Xie's team to selectively monitor fluctuations in sample absorption from the probe beam that accompany pump-mediated ground-state depletion. "It's a nonlinear technique that in principle allows us to do three-dimensional sectioning of a thick sample," he says, "because the signal only occurs at the laser focus where the intensity is the highest."

Sandoghdar's group devised an optimized configuration that allows them to directly monitor photon absorption from a single beam (Kukura *et al.*, 2010). "It comes down to whether the signal that you expect is larger than the intensity fluctuations that you have," he says. "You want your signal-to-noise ratio to be as large as possible." They achieved this by preparing samples under conditions that minimize scattering by virtually eliminating refractive-index heterogeneities; in parallel, a specialized photodetector configuration helped them compensate for random fluctuations in laser intensity. With this approach, they readily detected individual dye molecules.

None of these methods are currently suitable for single-molecule biological imaging, but they show immediate promise for imaging at slightly larger scales. "Photothermal applications are already there; it's a very efficient and sensitive technique and pretty simple to use," says Orrit. Xie hopes to use his method to detect clusters of biological chromophores, such as cytochromes within the mitochondrial membrane, and Sandoghdar sees applications for his technique in characterizing various nanoscale phenomena. "We're excited about looking at the diffusion of molecules or atoms on surfaces," he says. "This is something that people normally do with scanning tunneling or atomic force microscopes."

Above all, however, the hope is that these diverse routes to detection will stimulate further research in this area. "We all worked really hard on this and achieved it at the same time in three different ways," says Xie. "It's really good for the field."

Michael Eisenstein

RESEARCH PAPERS

Chong, S. *et al.* Ground-state depletion microscopy: detection sensitivity of single-molecule optical absorption at room temperature. *J. Phys. Chem. Lett.* **1**, 3316–3322 (2010).

Gaiduk, A. *et al.* Room-temperature detection of a single molecule's absorption by photothermal contrast. *Science* **330**, 353–356 (2010).

Kukura, P. *et al.* Single-molecule sensitivity in optical absorption at room temperature. *J. Phys. Chem. Lett.* **1**, 3323–3327 (2010).