

Here's looking at molecules

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The vision of a 'chemiscope' – a light-based microscope that looks at individual molecules and allows chemists to study specific bonds and even the orbitals within them – is a step closer to realisation. Michael Gross reports



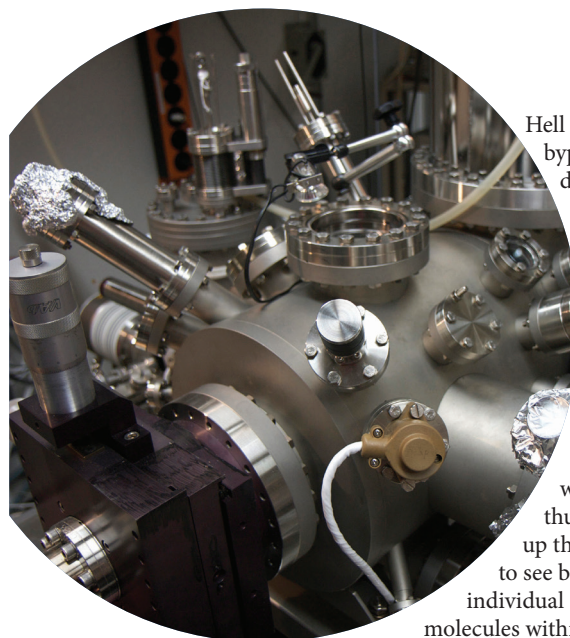
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Chemistry **C&I**

Light was in the focus of attention in the 2014 Nobel prizes for both chemistry and physics. While the physics award went to work that enabled the manufacture of blue LED lights, the chemistry prize honoured physical methods that enabled light microscopy on single molecules (*C&I*, 2014, 78, 11, 8).

Laureates William Moerner and Eric Betzig independently developed ways of observing the fluorescence of individual molecules of green fluorescent protein (GFP), the widely used molecular tag whose discovery and development was recognised by the Nobel committee only six years earlier. Physicist Stefan Hell from the Max Planck

Institute for Biophysical Chemistry at Göttingen, Germany, received his share of the 2014 honour for developing STED (stimulated emission depletion) microscopy, which sharpens the observable fluorescent spot by depleting the fluorescent states of surrounding molecules. Repeatedly applying this clever trick in the scanning mode,



Hell was able to bypass Abbe's diffraction limit (200nm) and obtain resolution well below the wavelength of light used, down to around 30nm.

The award winning work thus opened up the possibility to see beyond individual cells to large molecules within the cell.

While its applications would be mainly in biology rather than pure chemistry, work emerging now promises unprecedented microscopic methods that should be more useful to chemists. Questions such as 'Where exactly are the electrons in a molecule?' and 'How does a specific chemical bond form?' could soon find answers.

Towards the chemoscope

Ara Apkarian chairs the Center for Chemistry at the Space-Time Limit (CaSTL) at the University of California at Irvine, US. Launched in 2010, the centre aims to develop what Apkarian likes to call the 'chemoscope'. This, he explains, is a microscope that operates where chemistry happens – at the 0.1nm scale and on the femtosecond (10^{-15} s) timescale. He has a challenge on his hands, considering that Hell's Nobel-winning STED technology 'only' achieves a resolution of tens of nanometres and milliseconds.

CaSTL researchers are working on three different variations of the 'chemoscope'. Funded by the National Science Foundation, they have already succeeded in recording the motion of single electrons, single orbitals, and chemical bonds in single molecules. More recently, they have captured a single molecule changing its shape – an isomerisation reaction caught in the act.¹

Light in, light out

A traditional microscope is a purely optical instrument – light goes in,

interacts with the sample, and light comes out carrying information about the sample. One of the three chemiscopes fits this description.

A team led by Apkarian and Eric Potma used the all-optical chemoscope to capture the vibrations of a single molecule with femtosecond time resolution – a laser is used to interrogate the sample and light detectors record the output.² A video of the 'breathing' molecule they produced is published as an online supplement to their paper. To reach the required sensitivity, the researchers equipped the molecule with a nano-antenna consisting of two gold nanospheres, each 100nm in diameter, placed 1nm apart. By confining the light in a 1nm junction gap, the nano-antenna effectively focuses light down to the size of a molecule and dramatically enhances its local intensity.

This methodology is similar to that used in surface-enhanced Raman spectroscopy (SERS). Raman spectroscopy is based on light scattering from a vibrating molecule that changes not just the direction of the light but also its wavelength. As it is a fairly weak effect, it only became useful with the advent of powerful lasers and development of the enhancement technique; the latter relies on silver nanoparticles to amplify the Raman scattering signal and simultaneously quench any unwanted fluorescence background.

Richard Van Duyne of Northwestern University, US, who discovered the SERS effect in 1977, has been a member of CaSTL since its inception. He has recently led advances toward single molecule detection and space-time-resolved SERS, using picosecond and femtosecond lasers.

To obtain spatial resolution at the molecular scale, Van Duyne and his team combine SERS with a scanning tunnelling microscope (STM), where the junction between the STM tip and the substrate acts as the nano-antenna, thus replacing the gold nanoparticles of Apkarian and Potma's experiment. R. Zhang and colleagues at the University of Science and Technology of China at Hefei have shown that this tip-enhanced Raman spectroscopy

(TERS) can reach 1nm resolution.³ Indeed, it was using TERS that Apkarian's team was able to observe the isomerisation reaction of a single azobenzene molecule.¹

In another experiment, using a different combination of Raman and STM, where information is collected simultaneously from the STM and the Raman scattering, the researchers recorded sub-nanometre images of large organic molecules, including copper phthalocyanine, adsorbed onto metal surfaces. This investigation of chemical bonding was backed with theoretical calculations and simulated spectra.⁴ The researchers hope that this approach will deliver useful insights into the mechanisms of catalysis and other surface reactions, which in turn could lead to applications. Copper phthalocyanine, for example, has potential in optical and electronic applications, including light-emitting diodes and thin film transistors. The methods continue to be optimised and have recently proven highly efficient for investigating molecule-scale electronic effects.⁵

In a separate experiment using Raman spectroscopy as a nanoscale equivalent of GPS tracking, Apkarian's group showed that single molecules tagged with a suitable gold nanoparticle can be tracked in 3D space. The method can also serve to measure electric fields in the vicinity

of a molecule, providing detailed information that may help to elucidate reaction mechanisms.⁶

Electro-luminescence

Whereas the Raman-based methods use light both to look at the sample and for the read-out, there is also the possibility to interrogate the sample by injecting electrons. One such approach is based on the earlier work of Wilson Ho's group at Irvine, a charter member of CaSTL, who showed that light emission caused by tunnelling electrons in an STM experiment yield spectra

2015 Nobel chemistry prize recognised new methods for seeing beyond individual cells to large molecules within the cell



CaSTL researchers are developing three types of 'chemoscope' – microscopes that will operate at the 0.1nm scale and on the femtosecond timescale, exactly where and when chemistry happens



CaSTL researchers have already recorded the motion of single electrons, single orbitals and chemical bonds in single molecules, and an isomerisation reaction in real time



that can provide spatially resolved information about excited electronic states of a molecule in the angstrom range.⁷

Analysing the luminescence spectra of a magnesium porphyrin complex cooled to -270°C and immobilised on a thin film of alumina, the researchers showed that the light emission stimulated by the tunnelling electron comes from

Chemiscopes will lead to a better understanding of reaction mechanisms, improved catalysts for myriad industrial reactions, as well as new applications of molecules eg in nanoelectronics



the rim of this macrocyclic molecule, without any involvement of the central magnesium ion; information that could one day prove useful if porphyrins are to be used in nanoelectronic applications for example. In addition, the group was also able to collect information about the electronic states of the molecule by measuring spectra as a function of tip placement within the molecule.

As a step towards the goal of resolving chemistry as it happens in time and space, this experiment has yielded mechanistically detailed information at the required spatial resolution, although not yet in a time-resolved manner. However, Apkarian's group soon realised that line shapes in electroluminescence spectra retain phase information; which can be translated to time-dependent information through an established mathematical tool, the Fourier transformation. This yielded the first measurements with sub-molecular spatial and femtosecond time resolution, at the sought after Å-fs limit.⁸ 'The frames of the reconstructed movie show an electron orbiting the negatively charged molecule as it passes through, allowing a direct visualisation of what is otherwise known as shape resonances,' explains Apkarian.

In a separate experiment, also based on the luminescence method, Apkarian's group was able to observe a quantum mechanical phenomenon triggered by the presence of an unpaired electron in a negatively charged porphyrin ion – the so-

called Jahn-Teller effect. 'This is essentially a dynamical distortion of the molecular structure which drives the odd electron into a motion much like a hula hoop,' says Apkarian. We can use it to visualise the location and dynamics of the molecular frame and the orbiting orbital in which the electron resides.'

The orbital could be seen to have a cloverleaf structure when orbiting freely. The researchers were then able to suppress the motion by applying a voltage across the STM junction, upon which they could see the clover leaf collapse into a dumbbell shape.⁹ Such studies of the behaviour of individual electrons could help the quest to further miniaturise electronics. Building on this work, Apkarian says: 'We have taken the logical next step to demonstrate the ultimate limit in molecular electronics; by showing that the spin flip of one electron in one molecule can be used as a current switch with controllable frequency and sense of current flow.'¹⁰

Light in, force out

The third variant of the chemoscope relies on photo-induced force microscopy (PiFM), by using laser light input and atomic force microscopes (AFM) for the readout. This novel approach was invented by another member of CaSTL, Kumar Wickramasinghe,¹¹ who is one of the pioneers of AFM. His group developed the space-time resolved applications of PiFM in collaboration with Potma, with joint resolution of nanometre/picosecond demonstrated to date, and remarkably, under ambient laboratory conditions.¹²

Detecting optically induced forces instead of scattered light results in dramatic enhancements in sensitivity in stimulated Raman spectroscopy,¹³ along with all of the advantages of surface enhancement when using tips and junctions that can act as nano-antennas. Molecular Vista, a company that is closely affiliated with CaSTL, is currently developing a commercial instrument based on PiFM.

'In a short time, the lofty concept of the chemoscope has generated realisations that would have been difficult to anticipate at the inception

'Beyond its physical parts and pieces, the chemoscope involves a shift in paradigm in the way we interrogate the molecular world.'

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of the CaSTL Center some six years ago,' Apkarian notes. He adds that 'beyond its physical parts and pieces, the chemoscope involves a shift in paradigm in the way we interrogate the molecular world'.

Meanwhile, other laboratories are also keen on catching a glimpse of chemistry as it happens at the atomic scale. The group of Leo Gross at the IBM research centre at Zurich, Switzerland, for example, has combined STM and Kelvin probe force microscopy (KPFM), the latter involves doing the AFM measurement under different voltages. With this technique, the Zurich group has snapped a 'portrait' of an adsorbed pentacene molecule. In the most recent application of this method, the group of Jascha Repp at the University of Regensburg, Germany, together with researchers at Prague, Czech Republic, imaged the charge distribution (polarisation) in the bonds of an organic molecule.¹⁵

One may have philosophical objections to whether we can now really 'see' what's going on in chemical bonds, given the indirect nature of all these methods and the fundamental impossibility of seeing things smaller than the wavelength of light. Certainly, nobody will ever be able to peer down a tube containing a few lenses and watch chemical bonds forming and breaking.

And yet, there is no doubt that the efforts towards a 'chemoscope' are yielding visualisations of the fundamental units of chemistry, its atoms, orbitals and bonds, are both aesthetically appealing and intellectually rewarding. They will help nanotechnology's engineers to better understand and predict what they are doing at the atomic scale, and they enable all of us to see chemistry differently. ●

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