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Focusing on the details

Digital optical microscopy is set to answer fundamental questions in a range of fields. By **David Boothroyd**.

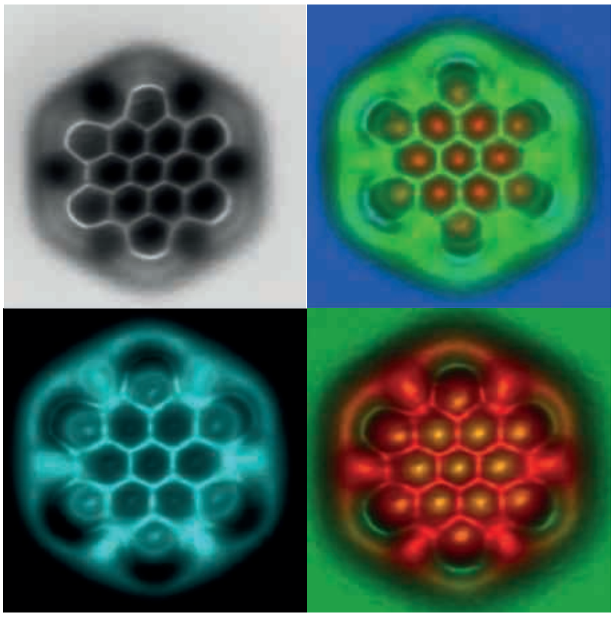
The microscope is one of science's oldest tools for examining nature, going back at least to the late 16th Century, with Galileo being its most famous pioneer – he called it the 'little eye'. For hundreds of years since, optical microscopes have revealed a world beyond our senses, pioneering huge areas of research and discovery. Today, there are scores of different forms of optical microscopy and one of the most valuable recent advances – the digital optical microscope – has been enabled by electronics. This uses a cmos sensor or ccd to convert light into electronic signals that can be displayed on a monitor, making eye pieces unnecessary.

But it is the use of particles other than photons – notably electrons – together with advances in electronics and other technologies, that has revolutionised microscopy over recent decades. It began in the 1930s with the development of the transmission electron microscope (TEM), which offers far greater resolution through the use of electrons, rather than light, and electromagnets, instead of glass lenses. The electron beam is passed through the sample being studied and the electrons are reflected or change direction. From this, an electron micrograph can be created.

The TEM was quickly followed in 1935 by the development of the scanning electron microscope (SEM). This represents another whole family of microscopes because it examines objects by scanning the surface with a fine electron beam as opposed to passing it through the sample. The beam are reflected and scattered and a 3d image is built up from this data.

Since then, a range of electron microscope techniques have been developed. The hallmark of them all is the extraordinary increase in resolution they provide – in the case of TEM, down to 0.05nm, for SEM, around 0.4nm, equivalent to a magnification factor of around 2million, and at least 1000 times greater than optical devices.

Another major branch of the microscope world is scanning force (or probe) microscopy, which comprises more than 20 different versions. One of the most widely used is a technology making important advances today, atomic force microscopy (AFM), capable of resolving to a fraction of a nanometre.



Images of a 1.4nm diameter hexabenzocoronene molecule captured using a non contact atomic force microscope

Since the first commercial device was introduced in 1989, AFM has become a key tool for imaging, measuring and manipulating matter at the nanoscale.

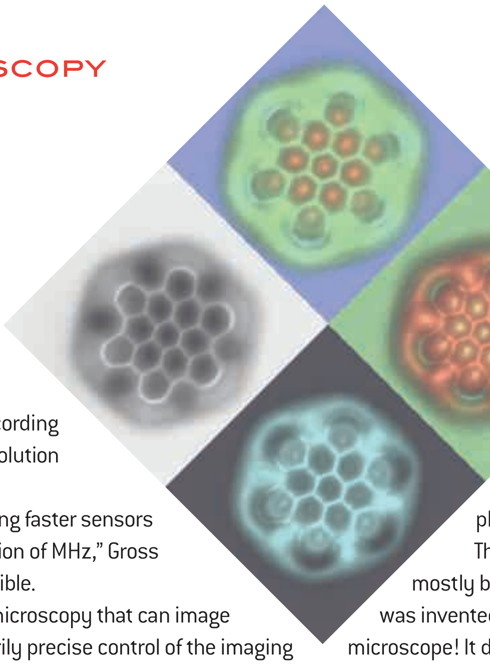
The AFM comprises a cantilever with a probe at its end with a radius measured in nanometres. This scans the surface of the material being studied. The cantilever is typically silicon or silicon nitride and piezoelectric elements make it possible to control the precise movements needed.

When the tip touches the sample, forces deflect the cantilever and from these deflections, read by piezoelectric sensors, an image can be built. AFM can study a whole range of forces, from basic mechanical contact force, to van der Waals forces, capillary forces, chemical bonding, electrostatic forces, magnetic forces and others. Usually, deflections are measured using a laser spot reflected from the top surface of the cantilever into an array of photodiodes, although other methods are used like optical interferometry or capacitive sensing.

A variation is non contact AFM (NC-AFM), in which there is no physical contact with the sample, a technique used by IBM Research Zurich. Here, a current is passed through the tip to probe the electrical conductivity of the underlying surface. The principles underpinning this go back to the beginning of the 1990s, when it was suggested that you could use frequency modulation (FM) signals, as Leo Gross, an IBM Research Staff Member, explains.

"You oscillate the cantilever, in our case a tuning fork, at the resonant frequency and as it gets close to the surface, but without touching it, it starts to get detuned. In the NC-AFM that IBM uses, the resonant frequency is around 30kHz and the shift is of just a few Hz, but this is enough to create an image by moving the tip of the cantilever – which consists of a single carbon monoxide (CO) molecule – across the sample." This makes it possible to image the atomic structure of the sample.

A recent achievement at IBM, using NC-AFM, has been the ability to differentiate the chemical bonds in a molecule, which differ in length by only 3picometres (3×10^{-12}m), or 1% of an atom's diameter. Bonds can be imaged and differentiated because they exhibit different electron densities, which show up in the images as areas of varying brightness. The results have

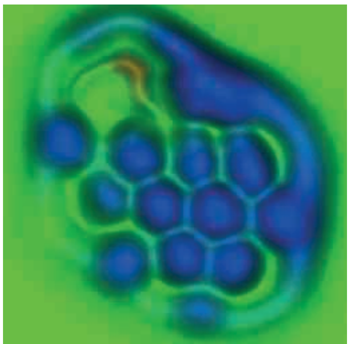


advanced the exploration of molecules and atoms at the smallest scale and could be important for studying applications such as graphene, organic solar cells and LEDs.

Despite its achievements, there are limitations to AFM. One is that it is slow – recording an AFM image of a molecule with atomic resolution takes around 30mins.

"We are working on speeding this up, using faster sensors with a higher resonant frequency; in the region of MHz," Gross says. Even real time video is becoming possible.

Another major challenge in any form of microscopy that can image individual atoms is the need for extraordinarily precise control of the imaging tip. This is made possible through the use of piezoelectric materials but also critical is very low temperature operation, down to 4K, achieved using liquid helium. IBM custom builds its systems, but makes use of commercially available components, from companies like SPS CreaTec.



Non contact atomic force microscopy highlights the different lengths and orders of the carbon-carbon bonds in this nanographene molecule

As well as increasing the speed of image capture, another potential advance for AFM technology is to widen the range of sensor tips used and the classes of molecules that are investigated, which could include biomolecules. And a different form of NC-AFM, called Kelvin probe force microscopy, is attracting a lot of attention. This uses electrostatic forces, as Gross explains.

"You apply a bias between tip and sample, sweep the bias across the sample and analyse how the force changes. This enables you to see charge differences within molecules that are even smaller than electron charges. This could be valuable to help the work going on with single electron devices, because it can show how single electron charges are distributed and moved within molecules."

For an instrument that we have been using for hundreds of years, the microscope is still proving to be a remarkable source of innovation. One recent development that owes its emergence to advanced IT is the digital holographic microscope. This aims to overcome limitations affecting many microscope techniques: a tiny field of view and a shallow depth of field. This makes it difficult to view objects where 3d information can be crucial, like living cells.

Answer: make a hologram of the sample. This is done in the usual way, by splitting a laser beam in two, then using one as a reference beam and reflecting the other off the sample to record the pattern of phase shifts that this produces. A digital sensor records the data. As with any hologram, recombining the beams produces an interference pattern that can be analysed by a reconstruction algorithm to build a 3d image of the sample.

The holographic technique not only records variations in the intensity of light bouncing off a sample, like conventional microscopy, but also phase information. Thanks to image processing software, this means you can change the depth of focus – effectively focusing after the image is recorded – and correct optical aberrations, as well as building the 3d image.

Another surprising advantage is that holographic microscopy can be low cost. Devices have been built for as little as $1000 and researchers at the Kisarazu National College of Technology in Japan have gone even further. Using a web camera, a small solid state laser, an optical pinhole and free open source software, they have cut the cost to $250.

What's more, there are several similar kinds of techniques, including interferometric microscopy, optical coherence tomography and diffraction phase microscopy. Common to all is the use of a reference wave front to obtain intensity and phase information.

There is a nice irony to this use of holography, which has so far mostly been applied to light microscopy. That is because holography was invented by Dennis Gabor in order to improve the electron microscope! It did not happen in his day, but there are signs that digital electron holography may finally work as Gabor hoped.

After photons and electrons, now we are seeing the emergence of neutrons as data for microscopy. A new neutron microscope called Larmor is to be built at the Rutherford Appleton Laboratory in Oxfordshire. By monitoring how neutrons are scattered by a sample, high precision images can be created. Since neutrons have no electrical charge, the beams can penetrate deeply into materials. Images with a resolution at the level of individual atoms should be achieved.

Neutron microscopy is suited to a range of applications, including observing magnetic materials, complex liquids, living biological specimens, and enhancing storage of charge in lithium ion batteries. Another possibility is studying new molecules that can transport medication to the exact location of a tumour.

It is not only microscope techniques and technology that are seeing surprising innovation: so too are their applications. One example is the use of an AFM by researchers at Zurich's ETH university to analyse a crystal that could tell us about the very early days of the cosmos, shortly after the Big Bang. A crystal of yttrium manganite was analysed by the AFM because of its 'multiferroic' behaviour, in which electric charges and magnetic dipoles arrange themselves spontaneously. The researchers discovered this arrangement of charges followed the same rules that describe the universe during its very early expansion.

Meanwhile, at the University of Berkeley and the National University of Singapore, a TEM is being used to manipulate nanoparticles. The TEM's electron beam traps gold nanoparticles and directs their movement, enabling the researchers to assemble several nanoparticles into a tight cluster. Also, because the beam is from an electron microscope, they can image the nanoparticles as they manipulate them.

Even the humble founder of it all, optical, is seeing advances, such as 'nonlinear' microscopy. A typical optical microscope is a linear instrument, meaning the atoms of a sample interact with only one photon at a time. This limits the ability to look below a surface. With a nonlinear microscope, a sample is examined using two intersecting, non parallel light rays. This makes it possible to capture images from beneath the sample's surface. A further innovation by Japanese researchers at the Riken Institute has enabled nonlinear optical techniques to resolve structures in mouse brains down to a depth of 240μm.