ELECTRON MICROSCOPY

The challenges of graphene

A study of nitrogen doping of graphene reveals the potential of high-resolution electron microscopy for imaging charge transfer around chemical bonds.

Knut W. Urban

The wealth of exceptional properties of graphene stimulates fundamental studies as well as the design of device applications. On the other hand, the material poses real challenges to established characterization techniques, providing an opportunity for improvement that can then be extended beyond this single layer of carbon atoms. For example, high-resolution transmission electron microscopy (HRTEM) can provide otherwise unattainable information on graphene if scientists are prepared to use their sophisticated instruments at their full capability.

An excellent example is provided by the work of Jannik Meyer and colleagues, who studied nitrogen doping of graphene, as reported in *Nature Materials*[1]. They provide experimental evidence for strong chemical-bonding-induced charge transfer from the carbon atoms into the region of the bond with the neighbouring nitrogen atom.

HRTEM has already contributed substantially to our knowledge of graphene. The computer simulation of a hexagonal grating of carbon atoms as a hill-and-valley landscape spreading to the horizon, which illustrates the electron microscopic observation that graphene is not ideally flat, is the way in which most people visualize this material[2]. Still, although the general structure of hexagonal rings of carbon can be seen by high-resolution microscopes, imaging the individual atoms and measuring their positions is not as straightforward as you might think[3]. It involves the reconstruction of the wavefunction of the imaging electron-wave field at the exit plane of the specimen from a series of images taken under precise conditions corresponding to specific settings of the objective lens focal distance. Information on the atomic structure is hidden in this wavefunction primarily in the form of a locally varying phase shift induced by the quantum-mechanical interaction with the atomic Coulomb potential, which has atom-core as well as electron components. The structure of graphene has only recently been imaged with true atomic resolution[4] by employing the wavefunction reconstruction technique in an aberration-corrected, state-of-the art instrument (see Fig. 1). The sensitivity achieved in that work was so high that the phase-shift difference between a carbon atom in the first and in the second layer of double-layer graphene could be measured. This allowed atomic depth resolution and made it possible to image and study carbon atoms in the two atom layers separately.

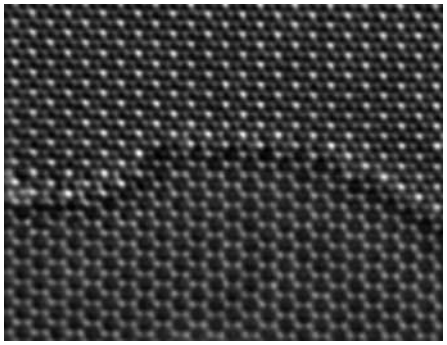

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Figure 1 | HRTEM image of single and bilayer graphene with atomic resolution.

Now, Meyer *et al.*[1] have demonstrated another leap forward in the contributions that HRTEM can make to our understanding of graphene's properties. Specifically, they used density-functional theory (DFT) to calculate the expected electron density around the nitrogen atom and used it to predict the atomic scattering potential for the electrons. By solving the Schrödinger equation for this potential, they predicted a local atomic-scale phase modulation in the electron wave, which they then observed as contrast in an aberration-corrected transmission electron microscope, providing evidence of the charge transfer mentioned above[5].

The study of local electronic structure, for example around defects, has so far largely been the domain of scanning tunnelling microscopy and spectroscopy[6], which are sensitive only to surfaces. More recently, atomically resolved electron-energy loss spectroscopy in the aberration-corrected scanning transmission electron microscope[7,8] has also been used: this, in contrast, is a volume technique in which a fine, high-intensity electron beam of angstrom or sub-angstrom diameter is scanned over a specimen, and the image is formed by recording the signal of a detector placed beneath the specimen. The work of Meyer *et al.* adds the transmission electron microscope equipped with state-of-the-art aberration-corrected electron optics to this short list of tools. We already know that the extraordinarily high signal-to-noise ratio of this kind of transmission electron microscopy can allow atomic picometre-precision microscopy[9]. Now we learn that it also provides a detection sensitivity of electron-wave phase changes so high that the subtle effects of chemical-bonding-induced charge transfer can be detected on the atomic level in the microscopic contrast.

Beyond this specific and important result, the work by Meyer *et al.* carries a message that electron microscopists should embrace fully if they want to take advantage of the new frontiers opened up by aberration-corrected electron optics: they have to reconsider two hitherto universally employed concepts. The first is the assumption of the 'independent-atom model'[10], according to which the scattering potential of a material is calculated as a superposition of atomic potentials originally computed for an isolated atom of every element. To a first approximation, this is reasonable because the adjustments to the potentials due to electron bonding effects are small and cannot be detected in conventional electron microscopy[11]. But it makes the sophistication of state-of-the-art aberration-corrected instruments pointless. For example, the independent-atom model does not predict any of the contrast effect observed by Meyer *et al.* for the nitrogen atom in the graphene lattice (Fig. 2). In the second example reported in the paper, single-layer hexagonal boron nitride, the independent-atom model predicts a substantial contrast difference which should make it possible to differentiate between the two types of atoms in the microscopic images. In reality, as shown by the DFT calculations, charge transfer in this partially ionic compound almost exactly cancels

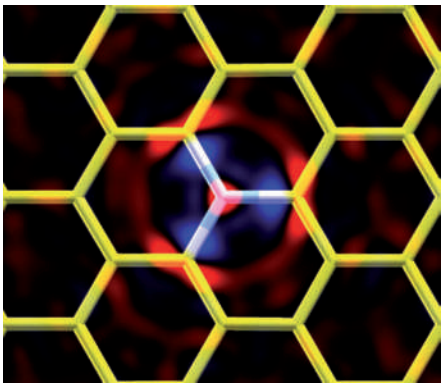

Figure 2 | DFT results for the changes in electron density due to bonding. The blue (red) colour shows a lower (higher) electron density than in the case in which the independent atomic model is used[1].

out the expected contrast difference. As a consequence, the two types of atom cannot be distinguished from each other, on physical grounds. This also shows that first-principle calculations should be used to take advantage of what sophisticated optics has to offer

The other popular concept in HRTEM is that of the 'ideally weak object'. As discussed in textbooks[12], a weak object is realized by a very thin sample consisting of atoms of low nuclear charge, which, owing to their weak electron scattering, induce only very weak phase shifts in the imaging electron-wave field. For such an idealized object, theory[13,14] yields phase-shift contrast that, as long as standard conditions are applied for acquiring an image, provides a direct representation of the scattering potential. This greatly aids image interpretation and has often served as an argument for deriving atomic-structure information from only a single acquisition, thus avoiding the painstaking effort needed to take image series and engage in complicated and time-consuming numerical procedures for electron-wavefunction reconstruction. Now, transmission electron microscopes do not transmit equally well all spatial frequencies that, in a Fourier representation, are required to describe an object structure correctly. For example, the microscope cuts spatial frequencies both in the high- and low-frequency range, thus acting as a low- and high-pass filter. Meyer and colleagues show that in the standard-conditions single acquisition mode, the contrast features induced by charge transfer are suppressed, making the bonding-induced effect unobservable. Yet single-layer graphene is, with respect to electron scattering, the weakest object so far available. Taking image series under defined variable-focus conditions allows expansion of the spatial-frequency characteristics of a microscope. This has to become part of the everyday routine of microscopy on the atomic scale.

To 'see' atoms and to measure the local electronic effect of integrating an atom into a solid is a long-standing dream in materials science. The recent work on graphene is a great step in this direction. In essence this means nothing less than an uncompromising application of quantum mechanics not only to the calculation of the electron structure in solids but also to the treatment of contrast formation in electron optics. ❐

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SYNTHETIC VACCINES

Immunity without harm

Multilamellar lipid vesicles with crosslinked walls carrying protein antigens in the vesicle core and immunostimulatory drugs in the vesicle walls generate immune responses comparable to the strongest live vector vaccines.

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Preventive (prophylactic) vaccines have greatly helped the global eradication of some infectious viral diseases, for example poliomyelitis or smallpox[1]. However, vaccines used against pathogens such as the HIV and the hepatitis C virus (HCV) have for the most part failed. One problem is that vaccines based on attenuated viruses, despite generating protective immunity[2,3], are frequently too toxic for clinical use. On the other hand, non-viral vaccines, which typically have an excellent toxicity profile, are relatively ineffective at promoting immunity. It is therefore necessary to develop a vaccination strategy that can generate effective immunity and have low toxicity. As reported in *Nature Materials*, Irvine and colleagues have achieved an important step in this direction[4]: a non-viral vaccine carrier that provides immune responses comparable to viral vectors[5].

The vaccine carriers are multilamellar vesicles (liposomes) with crosslinked bilayers that entrap protein antigens in the vesicle core, and immunostimulatory Toll-like receptor (TLR) ligands in between bilayers (TLR is a protein that recognizes structural patterns). Irvine and colleagues showed that the crosslinked liposomes act as a controlled-release reservoir of protein antigen and can also target dendritic cells *in vivo*. Dendritic-cell targeting is a key part of the multi-step process by which vaccines activate antigen-specific memory T cells[6] — cells that recognize and rapidly clear pathogens that caused previous infections. In this process (Fig. 1), dendritic cells pick up the injected liposomes and present the delivered antigen and immunostimulatory molecules to cytotoxic T cells. The dendritic cells secrete cytokines — cell-signalling protein molecules — that help activate T cells against the pathogen-specific antigens. These cells then proliferate and circulate through the body, most of them dying off within a few days. However, a small subset of the population of cytotoxic (or killer) T cells survives in the long term (even for decades), giving rise to memory T cells. On